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## PHYTOPLANKTON IN VIRGINIA LAKES AND RESERVOIRS

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### ABSTRACT

This study involves a phytoplankton summer/autumn survey in 46 Virginia lakes and reservoirs during 2010-2012. A total of 307 taxa were identified which included several filamentous and colonial cyanobacteria in bloom concentrations. With the exception of one natural lake, the other sites sampled represent impoundments created decades ago, with the majority presently classified as meso- or eutrophic. Among the cyanobacteria were 6 known toxin producers (*Anabaena circinalis*, *Anabaena spiroides*, *Aphanizomenon flos-aquae*, *Cylindrospermopsis raciborskii*, *Limnothrix redekei*, and *Microcystis aeruginosa*). The study characterizes phytoplankton populations in these aging freshwater habitats taken from a large number of sites over a broad geographical extent in Virginia. The results portend future concerns for increased presence of less favorable algal populations in Virginia lakes and reservoirs, including an increased occurrence of algal blooms, and presence of potential harmful species.

Keywords: Phytoplankton, lakes, reservoirs, Virginia

### INTRODUCTION

There are numerous water habitats designated as lakes in Virginia, and these vary greatly in size ranging from expansive reservoirs to what may be considered large ponds. The Virginia Department of Game and Inland Fisheries has identified ca. 150 of these sites commonly used for recreational activities (VDGIF 2002). In addition to these there are numerous other lakes and reservoirs of various sizes and recreational usage throughout Virginia. There are two natural lakes in Virginia (Lake Drummond and Mountain Lake), plus others representing man-made lakes and reservoirs formed by impoundments of rivers and streams. Waters from several of these sites are made palatable by water treatment facilities, with many commonly utilized for public recreational activities. There are also general concerns associated with both of these practices that relate to shifts in the composition of the algal populations in lakes over time. This transition involves changing from a more favorable assemblage of algae to those considered less desirable that will negatively influence water quality, even resulting in potentially harmful effects to animals and humans (Funari and Testai 2008, O'Neil et al. 2012). This change in algal composition typically accompanies the

transitional trophic changes in lakes as they age, with this pattern enhanced with increased nutrient input. These less favorable algae may be associated with taste and odor contamination to drinking water derived from these lakes, or associated with algal blooms and toxin production (Cronberg et al. 2003, Codd et al. 2005, Hoeger et al. 2005). Many of the Virginia lakes have reached, or are approaching the later trophic stages that favor development of these less desirable algal taxa. This study provides a current appraisal of phytoplankton composition from a broad selection of Virginia lakes and reservoirs, plus identifying the major bloom and potential toxin producing species in these waters.

There have been numerous freshwater algal studies in Virginia including Lewis et al. (1933) who identified 249 freshwater phytoplankton taxa from several streams and small swamps in the Piedmont region of Virginia. Forest (1954) identified 359 algae in the vicinity of Mountain Lake taken from ponds, creeks, soil, and rock habitats. Algal composition from a variety of regional sites within the James River Basin were extensively studied by Woodson (1959, 1960, 1962a, 1962b), Woodson and Holoman (1964, 1965), and Woodson and Prescott (1961). These collections primarily came from ponds, streams, and small swamps from this region, with Woodson and Holoman (1965) listing 631 algal species. A more restrictive survey based on chlorophytes and cyanobacteria was conducted by Nemeth (1969) in the two Virginia counties on the Delmarva Peninsula reporting 102 and 43 taxa respectively within these two categories. Palmer (1967) has also provided references mentioning algae from a variety of water habitats in Virginia. A comprehensive algal species list from Mountain Lake, including data from previous studies since 1930, was reported by Parson and Parker (1989). Their summary included 331 species from this lake with chlorophytes having the greatest representation with 180 taxa (54.3%), followed by 45 cyanobacteria (13.8%), 25 diatoms (7.5%), and other groups with lesser representation. There have also been numerous phytoplankton studies in Lake Drummond, a dystrophic lake in the Dismal Swamp of southeastern Virginia by Poore and Marshall (1972), Marshall (1976, 1979, 1990), and Phillips and Marshall (1993). Marshall (1979) identified 71 taxa from this natural lake which was dominated by diatoms and chlorophytes, with desmids a common component. Several of the other lakes in southeastern Virginia with phytoplankton listing include a borrow pit lake (Lake Trashmore, Sheavly and Marshall 1989), Hoffer Lake (Wolny 1999), plus the inclusion of Lake Kilby and Western Branch Reservoir (Muscio 2001).

To date there has been no general or recent survey of phytoplankton composition in Virginia lakes and reservoirs, or to address the degree of potentially harmful taxa that are present in these waters. Since the majority of these lakes were created several decades ago many of these have progressively advanced toward trophic conditions that are more favorable to algal bloom production by a less desirable algal community (e.g. cyanobacteria dominance). The objectives of this paper are: 1) to identify the present phytoplankton composition from a large representation of Virginia lakes and reservoirs, and 2) to note potentially harmful phytoplankton species in these waters.

#### METHODS AND MATERIALS

Water samples for phytoplankton analysis came from 46 Virginia lakes and reservoirs (Fig. 1). Collections were made July through September over 3 years (2010-2012). Surface (<1m) water samples (500-1000 ml) were collected and preserved with



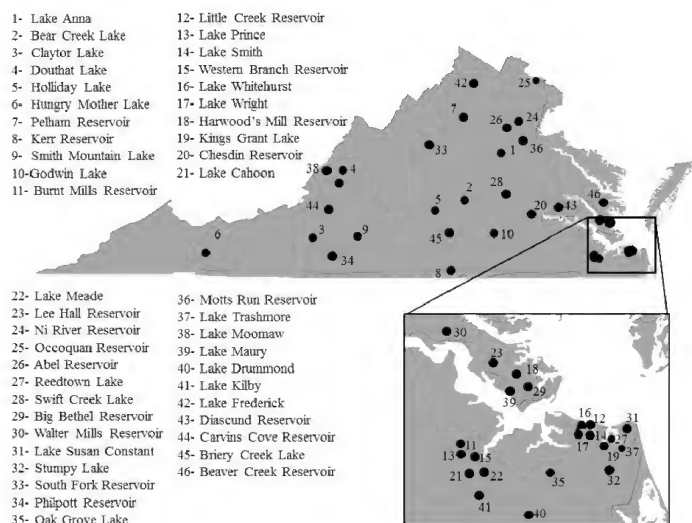


FIGURE 1. Locations of 46 Virginia lakes and reservoirs where phytoplankton samples were collected for analysis, 2010-2013.

Lugol's solution (1-2 ml). The most extensive collections were in 2012 when water samples were taken from 40 of the lakes and reservoirs. Data from 6 additional lakes from 2010 and 2011 are also included. An Utermöhl method of analysis using an inverted plankton microscope was used to examine concentrated aliquots of these samples at magnifications of 300X and 600x (Marshall 1976). Cyanobacteria filaments were counted as numbers per ml, with colonial and unicellular cyanobacteria, and the other algal groups counted as number of cells per ml.

## RESULTS AND DISCUSSION

The lakes and reservoirs sampled came from several of Virginia's geographical regions. These were the Coastal Plain, Piedmont, Blue Ridge Mountains, and the Valley/Ridge. There were considerable differences among the lakes sampled regarding surface area, their age, depth, and elevation. The majority of these were impoundments created decades ago and have now progressed to later more nutritive levels of development. The trophic status for 39 of the 46 lakes was determined by VWCB (1992) using water quality values, with another 4 by the author based on their bloom and compositional status. The other 3 were not designated to a specific status due to lack of nutrient information. Based on these evaluations of the 43 sites, 25 (58.1%) were considered eutrophic, 12 (27.9%) mesotrophic, and 5 (11.6%) oligotrophic lakes, with 1 (2.3%) dystrophic lake. Thus, of these lakes 85% were in either a mesotrophic

or eutrophic stage of development. In addition, 23 of these also represented a public water supply source. Depths for many were < 4 meters, while the greatest depths came from Kerr Reservoir (ca. 30m), with a mean depth of ca. 9.1m. Ten of the lakes (21.7%) had a surface area of <40 ha, with 18 (39.1%) a surface area ca. 40-200 ha, 7 lakes (15.2%) at ca. 200-400 ha, and 9 lakes (19.7%) ca. 400-4047 ha, plus the two largest sites (4.3%) were Kerr Reservoir and Smith Mountain Lake, with surface areas of ca. 19,790 and 8,336 ha respectively. Lake Godwin was among the smallest in area at ca. 6 ha. During these collections the surface (<1m) temperatures ranged from 19.6 to 33.3°C, with an average of 27.1°C.

A total of 307 phytoplankton taxa were identified (Table 1). They consisted of 126 chlorophytes (41.0%), 78 cyanobacteria (25.4%), 44 diatoms (14.3%), 38 euglenophytes (12.9 %), 10 dinoflagellates (3.2%), 5 cryptophytes 1.6%), 5 chrysophytes (1.6%), and 1 raphidophyte (<0.01%). There were also several unidentified, but not common chlorophytes and pennate diatoms among the samples. The general algal flora was characteristic for U.S. north temperate fresh water lakes (Prescott 1951). However, since the collections represent a limited series of collections (3 months of the year), a more comprehensive seasonal series of observations, plus additional sampling sites, would likely provide additional taxa and expand the species list. In this study, the greatest concentrations of algae and algal bloom development were associated with colonial and filamentous cyanobacteria. The sites having a more advanced trophic status also had a greater and more abundant representation of these cyanobacteria, and included Lake Maury, Little Creek Reservoir, Lake Whitehurst, and Pelham Reservoir. The most abundant cyanobacteria at these locations were: *Anabaena* sp., *Aphanozomenon flos-aquae*, *Chroococcus dispersus*, *Cylindrospermopsis raciborskii*, *Glaucospira laxissima* (*Spirulina laxissima*), *Jaaginema metaphyticum* (*Oscillatoria angusta*), *Limnothrix redekei* (*Oscillatoria redekei*), *Merrismopedia tenuissima*, *Planktolyngbya contorta* (*Lyngbya contorta*), *Planktolyngbya limnetica* (*Lyngbya limnetica*), *Planktolyngbya* cf. *minor* (*Lyngbya limnetica* f. *minor*), *Pseudanabaena limnetica* (*Oscillatoria limnetica*), and *Woronichinia naegelianiana*. The lakes having a lower trophic status (e.g. dystrophic, oligotrophic) contained a greater representation of chlorophytes and diatoms, with lower phytoplankton abundance and less diversity. Among these sites were: Abel Reservoir, Hungry Mother Lake, Douthat Lake, Lake Kilby, Lake Drummond, and Carvins Cove Reservoir. The common chlorophytes at these and the other sites included *Ankistrodesmus falcatus*, *Crucigenia tetrapedia*, *Closteriopsis longissima*, *Desmodesmus quadricauda*, *Monoraphidium contortum*, *Scenedesmus bijuga*, and *Staurostrum* sp. Diatoms frequently present were *Aulacoseira granulata*, *Fragilaria crotonensis*, and a variety of pennate species. By using the concentrations of the various species at all of these lakes during the 2012 collections (e.g. 40 sites), the mean representation percentage in abundance among the major algal categories was: cyanobacteria (71.2%), chlorophytes (13.7%), diatoms (9.4%), cryptophytes (3.5%), euglenophytes (0.6%), raphidophytes (0.3%), chrysophytes (0.2%), and dinoflagellates (0.1%). A contributing factor regarding the high cyanobacterial abundance were their blooms during this period at 9 (22.5%) of the 40 sites sampled in 2012.

These 9 cyanobacteria blooms occurring during the 2012 collections, with their abundance maxima, were: *Chroococcus dispersus* (85,215 cells/ml), *Chroococcus minutus* (20,093 cells/ml), *Jaaginema subtile* (12,259 filaments/ml), *Jaaginema*

TABLE 1. Phytoplankton species from water samples taken in July, August, and September 2010-2012 from 46 Virginia lakes and reservoirs.

**Chlorophytes**

<i>Actinastrum hantzschii</i> Langerheim	<i>Desmodesmus armatus</i> (Chodat) Hegewald
<i>Actinastrum gracilimnia</i> G.M. Smith	<i>Desmodesmus armatus</i> v. <i>longispina</i> (Chodat) Hegewald
<i>Ankistrodesmus falcatus</i> (Corda) Ralfs	<i>Desmodesmus brasiliensis</i> (Bohlin) Hegewald
<i>Ankistrodesmus falcatus</i> v. <i>acicularis</i> (Braun) G.S. West	<i>Desmodesmus maximus</i> (W.& G.S. West) Hegewald
<i>Ankistrodesmus falcatus</i> v. <i>mirabilis</i> (West et West) G.S. West	<i>Desmodesmus opoliensis</i> (Richter) Hegewald
<i>Ankistrodesmus nannoselene</i> Skuja	<i>Desmodesmus quadricauda</i> (Turpin) Hegewald
<i>Botryococcus sudeticus</i> Lemmermann	<i>Desmodesmus</i> sp.
<i>Botryococcus braunii</i> Kützing	<i>Dictyosphaerium pulchellum</i> Wood
<i>Centritractus belenophorus</i> Lemmermann	<i>Dimorphococcus lunatus</i> A. Braun
<i>Chlamydomonas globosa</i> Snow	<i>Eudorina elegans</i> Ehrenberg
<i>Chlamydomonas gloeogama</i> Korshikov	<i>Franceia</i> sp.
<i>Chlamydomonas reinhardtii</i> Dangard	<i>Golenkiniopsis</i> sp.
<i>Chlamydomonas</i> sp.	<i>Gonium pectoral</i> O.F. Müller
<i>Chlorella ellipsoida</i> Gerneck	<i>Kirchneriella contorta</i> (Schmidle) Bohlin
<i>Chlorella minutissima</i> Fott et Novakova	<i>Kirchneriella lunaris</i> (Kirchner) Möbius
<i>Chlorella vulgaris</i> (Beyerinck) Beijerinck	<i>Kirchneriella lunaris</i> v. <i>irregularis</i> G.M. Smith
<i>Chlorococcum echinogyotum</i> Starr	<i>Kirchneriella obesa</i> v. <i>major</i> (Bernard) G.M. Smith
<i>Chlorococcum humicola</i> (Nägeli) Rabenhorst	<i>Lagerheimia</i> sp. Chodat
<i>Chlorococcum infusionum</i> (Schrack) Meneghini	<i>Lauterborniella elegantissima</i> Schmidle
<i>Chloromonas tenbraria</i> (Skuja) Gerloff et Ettl	<i>Micractinium pusillum</i> Fresenius
<i>Closteriopsis acicularis</i> (G. M. Smith) Belcher et Swale	<i>Microspora pachyderma</i> (Wille) Lagerheim
<i>Closteriopsis longissima</i> (Lemmermann) Lemmermann	<i>Monoraphidium arcuatum</i> (Korshikov) Hindák
<i>Coelastrum microsporum</i> Nägeli	<i>Monoraphidium contortum</i> (Thuret) Komárková-Legnerová
<i>Coelastrum reticulum</i> (Dangeard) Senn	<i>Monoraphidium irregulare</i> (G.M. Smith) Komárková- Legnerová
<i>Coelastrum sphaericum</i> Nägeli	<i>Mougeotia</i> sp.
<i>Crucigenia fenestrata</i> Schmidle	<i>Oedogonium</i> sp.
<i>Crucigenia tetrapedia</i> (Kirchner) W. et G.S. West	
<i>Crucigenia truncata</i> G.M. Smith	
<i>Crucigeniella crucifera</i> (Wolle) Komárek	

TABLE 1. Continued.

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<i>Oocystella borgei</i> J. Snow	<i>Tetraedron incus</i> (Teiling) G.M. Smith
<i>Oocystis elliptica</i> West	<i>Tetraedron minimum</i> (Braun) Hansgirg
<i>Oocystis lacustris</i> Chodat	<i>Tetraedron regulare</i> Kützing
<i>Oocystis marssonii</i> Nägeli	<i>Tetraedron trigonum</i> v. <i>gracile</i> (Reinsch) Detoni
<i>Pandorina morum</i> (Müller) Bory de Saint-Vincent	<i>Tetrahedron muticum</i> (A. Braum) Hansgirg
<i>Pediastrum biradiatum</i> Meyen	<i>Trichonema bourrellyi</i> (J.W.G. Lund) Anagnostidis
<i>Pediastrum duplex</i> Meyen	<i>Treubaria setigerum</i> (Archer) G.M. Smith
<i>Pediastrum duplex</i> v. <i>gracillimum</i> West & G.S. West	<i>Ulothrix</i> sp.
<i>Pediastrum obtusum</i> Lucks	<i>Westella linearis</i> G.M. Smith
<i>Pediastrum simplex</i> (Meyen) Lemmermann	<i>Zygnema</i> sp.
<i>Pediastrum tetras</i> (Ehrenberg) Ralfs	<b>Chlorophytes: Desmidiaceae</b>
<i>Pediastrum tetras</i> v. <i>tetraodon</i> (Corda) Rabenhorst	<i>Arthrodesmus incus</i> v. <i>extensus</i> Anderson
<i>Quadrigula closterioides</i> (Bohlin) Printz	<i>Closterium gracile</i> Brébisson
<i>Quadrigula lacustris</i> (Chodat) G.M. Smith	<i>Closterium lineatum</i> Ehrenberg
<i>Rhizoclonium hieroglyphicum</i> (C.A. Agardh) Kützing	<i>Closterium rostratum</i> Ehrenberg
<i>Scenedesmus acuminatus</i> (Lagerheim) Chodat	<i>Closterium setaceum</i> Ehrenberg
<i>Scenedesmus bijuga</i> (Turpin) Lagerheim	<i>Closterium</i> sp.
<i>Scenedesmus denticulatus</i> Lagerheim	<i>Cosmarium circulare</i> Reinsch
<i>Scenedesmus dimorphus</i> (Turpin) Kützing	<i>Cosmarium portianum</i> Archer
<i>Scenedesmus incrassatulus</i> Bohlin	<i>Cosmarium</i> sp.
<i>Scenedesmus opoliensis</i> P. Richter	<i>Desmidium aptogonum</i> Brébisson
<i>Scenedesmus</i> sp. Meyen	<i>Desmidium</i> sp.
<i>Schroederia setigera</i> (Schröder) Lemmermann	<i>Desmidium swartzii</i> Agardh
<i>Selenastrum bibraianum</i> Reinsch	<i>Euastrum bidentatum</i> Nägeli
<i>Selenastrum minutum</i> (Nägeli) Collins	<i>Euastrum divaricatum</i> Lundell
<i>Selenastrum westii</i> G.M. Smith	<i>Gonatozygon kinahani</i> (Archer) Rabenhorst
<i>Spirogyra</i> sp.	<i>Hyalotheca disseliens</i> (Smith) Brébisson
<i>Tetraedron enorme</i> (Ralfs) Hansgirg	<i>Micrasterias radata</i> Hassall
<i>Tetraedron gracile</i> (Reinsch) Hansgirg	<i>Micrasterias</i> sp.
	<i>Penium margaritaceum</i> (Ehrenberg) Brébisson
	<i>Spirotaenia condensata</i> Brébisson

TABLE 1. Continued.

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<i>Staurastrum paradoxum</i> Meyen	<i>Eunotia pectinalis</i> (Kützing)
<i>Staurastrum americanum</i> (West et West) G.M. Smith	Rabenhorst
<i>Staurastrum chaetoceras</i> (Schröder) G.M. Smith	<i>Eunotia</i> sp.
<i>Staurastrum cingulum</i> (West et G.S. West) G.M. Smith	<i>Fragilaria capucina</i> Desmarzières
<i>Staurastrum leptocladum</i> Nordstedt	<i>Fragilaria crotenensis</i> Kitton
<i>Staurastrum manfeldtii</i> Delponte	<i>Fragilaria crotenensis</i> v. <i>oregona</i> Sovereign
<i>Staurastrum obiculare</i> Ralfs	<i>Fragilaria</i> sp.
<i>Staurastrum paradoxum</i> Meyen	<i>Frustulia rhomboids</i> (Ehrenberg) DeToni
<i>Staurastrum quadricuspedatum</i> Turner	<i>Gyrosigma</i> sp.
<i>Staurastrum</i> sp.	<i>Meridion circulare</i> (Greville) C. Agardh
<i>Triplocerus gracile</i> Bailey	<i>Navicula</i> sp.
<i>Xanthidium</i> sp. Ehrenberg	<i>Neidium iridis</i> v. <i>vernalis</i> Reichelt
	<i>Nitzschia acicularis</i> W. Smith
	<i>Nitzschia</i> sp.
<b>Diatoms</b>	<i>Pinnularia acuminata</i> W. Smith
<i>Asterionella formosa</i> Hassal	<i>Pinnularia biceps</i> Gregory
<i>Asterionella formosa</i> v. <i>ralfsii</i> (W. Smith) Wolle	<i>Pinnularia gibba</i> Ehrenberg
<i>Aulocosiera distans</i> (Ehrenberg) Simonsen	<i>Pinnularia lata</i> (Brébisson) Rabenhorst
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	<i>Pinnularia latevittata</i> Cleve
<i>Aulacoseira granulata</i> v. <i>angustissima</i> (O. Müller) Simonsen	<i>Pinnularia</i> sp.
<i>Aulacoseira herzogii</i> (Lemmermann) Simonsen	<i>Pleurosigma angulatum</i> (Queckett) W. Smith
<i>Aulacoseira islandica</i> (O. Müller) Simonsen	<i>Pleurosigma</i> sp.
<i>Cocconeis</i> sp.	<i>Surirella biseriata</i> v. <i>constricta</i> (Ehrenberg) Grunow
<i>Cyclotella caspia</i> Grunow	<i>Surirella</i> sp.
<i>Cyclotella meneghiniana</i> Kützing	<i>Synedra acus</i> Kützing
<i>Cyclotella</i> sp.	<i>Synedra delicatissima</i> W. Smith
<i>Cyclotella stelligera</i> Cleve et Grunow	<i>Synedra radians</i> Kützing
<i>Cymbella ehrenbergii</i> Kützing	<i>Synedra ulna</i> f. <i>lingissima</i> (W. Smith) Brun
<i>Epithemia turgida</i> (Ehrenberg) Kützing	<i>Tabellaria fenestrata</i> (Lyngbye) Kützing
<i>Eunotia curvata</i> (Kützing) Lagerheim	<i>Tabellaria flocculosa</i> (Roth) Kützing
	<b>Euglenophytes</b>
	<i>Euglena acus</i> Ehrenberg
	<i>Euglena caudata</i> Hübner
	<i>Euglena convoluta</i> Korshikov

TABLE 1. Continued.

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<i>Euglena deses</i> Ehrenberg	<i>Trachelomonas</i> sp.
<i>Euglena ehrenbergii</i> Klebs	<i>Trachelomonas superba</i> Swirenko
<i>Euglena mutabilis</i> Schmitz	<i>Trachelomonas volvocina</i> Ehrenberg
<i>Euglena oxyuris</i> Schmarda	
<i>Euglena proxima</i> Dangeard	<b>Cryptophytes</b>
<i>Euglena</i> sp.	<i>Chroomonas</i> sp.
<i>Euglena spirogyra</i> Ehrenberg	<i>Cryptomonas erosa</i> Ehrenberg
<i>Gonyostomum</i> sp.	<i>Cryptomonas marssonii</i> Skuja
<i>Leptocinclis ovum</i> (Ehrenberg)	<i>Cryptomonas ovata</i> Ehrenberg
Lemmermann	<i>Cryptomonas</i> sp.
<i>Leptocinclis</i> sp.	
<i>Phacus acuminatus</i> Stokes	<b>Raphidophyte</b>
<i>Phacus caudatus</i> Hübner	<i>Gonyostomum semen</i> (Ehrenberg)
<i>Phacus curvicauda</i> Swirenko	Diesing
<i>Phacus lemmermannii</i> (Swirenko)	
Skvortsov	<b>Chrysophytes</b>
<i>Phacus longicauda</i> (Ehrenberg)	<i>Dinobryon bavericum</i> Imhof
Dujardin	<i>Dinobryon divergens</i> Imhof
<i>Phacus obicularis</i> Hübner	<i>Dinobryon sertularia</i> Ehrenberg
<i>Phacus</i> sp.	<i>Mallomonas acaroids</i> Perty
<i>Phacus suecicus</i> Lemmermann	<i>Synura caroliniana</i> Whitford
<i>Phacus tortus</i> (Lemmermann)	
Skvortsov	<b>Dinoflagellates</b>
<i>Phacus undulatus</i> (Skvortsov)	<i>Ceratium cornutum</i> (Ehrenberg)
Pochmann	Claparède et Lachmann
<i>Strombomonas longicauda</i>	<i>Ceratium hirundinella</i> (Müller)
(Swirenko) Deflandre	Dujardin
<i>Trachelomonas acanthophora</i> Stokes	<i>Gymnodinium</i> sp.
<i>Trachelomonas acanthostoma</i> Stokes	<i>Peridinium aciculiferum</i>
<i>Trachelomonas alisoviana</i> Skvortsov	Lemmermann
<i>Trachelomonas armata</i> (Ehrenberg)	<i>Peridinium bipes</i> Stein
Stein Lemmermann	<i>Peridinium inconspicuum</i>
<i>Trachelomonas gibberosa</i> Playfair	Lemmermann
<i>Trachelomonas globularis</i> v. <i>boyeri</i>	<i>Peridinium pusillum</i> (Lenard)
(Palmer) Conrad	Lemmermann
<i>Trachelomonas hispida</i> (Perty) Stein	<i>Peridinium</i> sp.
<i>Trachelomonas hispida</i> v. <i>coronata</i>	<i>Peridinium westii</i> Lemmermann
Lemmermann	<i>Peridinium wisconsinense</i> Eddy
<i>Trachelomonas intermedia</i> Dangeard	
<i>Trachelomonas raciborskii</i>	<b>Cyanobacteria</b>
Woloszyńska	<i>Anabaena affinis</i> Lemmerman
<i>Trachelomonas similis</i> Swirenko	<i>Anabaena circinalis</i> Rabenhorst

TABLE 1. Continued.

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<i>Anabaena perturbata</i> v. <i>tumida</i> (Nygaard) Cronberg et Komárek	<i>Coelosphaerium kuetzingianum</i> Nägeli
<i>Anabaena planctonica</i> Brunthaler	<i>Cylindrospermopsis philippinensis</i> (Taylor) Komárek
<i>Anabaena spiroides</i> Klebahn	<i>Cylindrospermopsis raciborskii</i> (Woloszyńska) Seenayya et Subba Raju
<i>Anabaena</i> sp.	<i>Dactylococcus acicularis</i> Lemmermann
<i>Anabaena viguieri</i> Denis et Frémy	<i>Dactylococcus fascicularis</i> Lemmermann
<i>Anabaena wisconsinense</i> Prescott	<i>Dactylococcus raphidiodes</i> Hansgrig
<i>Anabaenopsis elenkinii</i> Muller	<i>Dactylococcus</i> sp.
<i>Aphanizomenon flos-aquae</i> (L.) Ralfs	<i>Geitlerinema amphibium</i> (Agardh) Anagnostidis
<i>Aphanizomenon gracile</i> (Lemmermann) Lemmermann	<i>Glaucospira agilissima</i> Lagerheim
<i>Aphanizomenon issatschenkoi</i> (Ussaczew) Proschkina- Laurenko	<i>Glaucospira laxissima</i> G.S. West
<i>Aphanocapsa delicatissima</i> W. et G.S. West	<i>Gleocapsa punctata</i> Nägeli
<i>Aphanocapsa elaschista</i> W. et G.S. West	<i>Gleocapsa</i> sp.
<i>Aphanocapsa incerta</i> (Lemmermann) Cronberg et Komárek	<i>Gomphosphaeria aponina</i> Kützing
<i>Aphanocapsa pulchra</i> (Kützing) Rabenhorst	<i>Jaaginema metaphyticum</i> Komárek
<i>Aphanothece clathrata</i> W. et. G.S. West	<i>Jaaginema neglectum</i> (Lemmermann) Anagnostidis et Komárek
<i>Aphanothece minutissima</i> (W. West) Komárková-Legnerová et Cronberg	<i>Jaaginema subtilissimum</i> (Kützing) Anagnostidis et Komárek
<i>Aphanothece nidulans</i> Richter	<i>Limnothrix planctonica</i> (Woloszyńska) Meffert
<i>Chroococcus dispersus</i> (Keissler) Lemmermann	<i>Limnothrix redekei</i> (Van Goor) Meffert
<i>Chroococcus limneticus</i> Lemmermann	<i>Lyngbya birgei</i> G.M. Smith
<i>Chroococcus minor</i> (Kützing) Nägeli	<i>Lyngbya major</i> Meneghini
<i>Chroococcus minutus</i> (Kützing) Nägeli	<i>Merismopedia elegans</i> A. Braun
<i>Chroococcus pallidus</i> (Nägeli) Nägeli	<i>Merismopedia glauca</i> (Ehrenberg) Kützing
<i>Chroococcus</i> sp.	<i>Merismopedia punctata</i> Meyen
<i>Chroococcus turgidus</i> (Kützing) Nägeli	<i>Merismopedia tenuissima</i> Lemmermann
	<i>Microcystis aeruginosa</i> (Kützing) Kützing
	<i>Microcystis incerta</i> Lemmermann

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TABLE 1. Continued.

<i>Microcystis smithii</i> Komárek et Anagnostidis	<i>Pseudanabaena acicularis</i> (Nygard) Anagnostidis et Komárek
<i>Microcystis viridis</i> (A. Braun) Lemmermann	<i>Pseudanabaena limnetica</i> (Lemmermann) Komárek
<i>Nostoc commune</i> Vaucher	<i>Raphidiopsis curvata</i> Fritsch
<i>Nostoc</i> sp.	<i>Rhabdoderma lineare</i> Schmidle et Lauterborn
<i>Oscillatoria limosa</i> Agardh	<i>Rhabdogloea smithii</i> (R. et F. Chodat) Komárek
<i>Oscillatoria princeps</i> Vaucher	<i>Snowella lacustris</i> (Chodat) Komárek et Hindák
<i>Oscillatoria</i> sp.	<i>Snowella litoralis</i> (Häyerén) Komárek et Hindák
<i>Phormidium</i> sp.	<i>Spirulina laxa</i> G.S. Smith
<i>Planktolyngbya contorta</i> (Lemmermann)	<i>Spirulina meneghiniana</i> Zanardini
Anagnostidis et Komárek	<i>Spirulina subsalsa</i> Oersted
<i>Planktolyngbya limnetica</i> (Lemmermann)	<i>Spirulina weissii</i> Drouet
Komárková-Legnerová et Cronberg	<i>Synechococcus</i> sp.
<i>Planktolyngbya minor</i> (Geitler) Komárek et Cronberg	<i>Trichodesmium lacustre</i> Klebahn
<i>Planktothrix agardhii</i> (Gomont) Anagnostidis et Komárek	<i>Woronichinia naegeliana</i> (Unger) Elenkin

*metaphyticum* (19,315 filaments/ml), *Microcystis aeruginosa* (43,056 cells/ml), *Planktolyngbya contorta* (13,933 filaments/ml), *Planktolyngbya limnetica* (39,169 filaments/ml), *Pseudanabaena limnetica* (41,860 filaments/ml), and *Woronichinia naegeliana* (472,056 cells/ml). Another algal bloom occurring at one site during this time period was by the chlorophyte *Rhizoclonium hieroglyphicum* (2,332 filaments/ml). The number of cells in the algal filaments varied greatly within and between species (e.g. 8-38 cells/filament). In contrast to the cyanobacteria blooms that were more common in the open expanse of these waters, the *R. hieroglyphicum* bloom occurred in a narrow and shallow (<2m) extension of the eutrophic Lake Smith. *Ceratophyllum demersum* (Hornwort), an aquatic angiosperm, provided an extensive submerged growth that extended beneath and intermingled with the *R. hieroglyphicum* filaments. This was a long lasting bloom that was noted in early August and continued into November 2012.

Included among the phytoplankton were 6 species known to be potential toxin producers (Cronberg et al. 2003). These were *Anabaena circinalis*, *Anabaena spiroides*, *Aphanizomenon flos-aquae*, *Cylindrospermopsis raciborskii*, *Limnothrix redekei*, and *Microcystis aeruginosa*. Of the 46 lakes sampled 27 (58.6%) contained at least 1 of these species, with 19 (41.4 %) sites not having any during these collections.



*Aphanizomenon flos-aquae* was in 14 (30.4%) of the lakes, with *Limnotherix redekei* in 10 (21.7%), and *Cylindrospermopsis raciborskii* in 7 (15.2%) of the lakes and reservoirs. Eleven (23.9%) of the lakes had 2-3 of these HABs, and only Lake Prince had 4 (*A. circinalis*, *A. spiroides*, *A. flos-aquae*, *C. raciborskii*). During these collections none of these taxa attained major bloom status, nor were there any known fish kills or harmful impacts associated with these algae.

#### SUMMARY

A total of 307 freshwater algae were identified from 46 Virginia lakes and reservoirs. The majority of these locations represented advanced (meso-eutrophic) stages of development that provided favorable conditions for algal diversity and growth. Of note was the abundance of several bloom-producing filamentous and colonial cyanobacteria, including 6 potentially harmful species (with at least one of these in over half of the sites sampled). These data indicate there are numerous freshwater sites in Virginia among those sampled here that are favorable locations for bloom producing algae, including the less desirable species associated with degraded water quality and toxin production.

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## The Population Dynamics of Two Rodents in Two Coastal Marshes in Virginia

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### ABSTRACT

The communities of small mammals were evaluated for 13 months with capture-mark-recapture methods in two *Spartina-Juncus* marshes of the Atlantic coast in Northampton County, Virginia. Small mammals were trapped for three days each month using live traps placed on floats on two study grids. Two rodents were numerically dominant (~90% of small mammals) there: marsh rice rat, *Oryzomys palustris*, and meadow vole, *Microtus pennsylvanicus*. Monthly estimates of population density were greater for rice rats (peak: 45/ha) than for those of meadow voles (peak: 30/ha). Survival rates were generally low, especially for rice rats, indicating highly vagile populations. Both species had greatest breeding activity in spring and autumn, with lower rates in summer and winter. Sex ratios favored males in rice rats but were unity in meadow voles. Although marsh rice rats, being semi-aquatic and capable swimmers, are more highly adapted to living in flooded marsh environments, meadow voles can thrive there too.

### INTRODUCTION

Two species of rodent, marsh rice rat, *Oryzomys palustris*, and meadow vole, *Microtus pennsylvanicus*, are dominant in the marshes of the coast and the nearby barrier islands in eastern Virginia (Bloch and Rose 2005, Cranford and Maly 1990, Dueser et al. 1979). These species have been frequently studied elsewhere, but rarely together because the meadow vole is a boreal species near its southern limit in eastern Virginia and *Oryzomys*, a tropical genus, is widespread only from coastal Delaware southward.

Early studies reporting the presence of the marsh rice rat in Virginia tidal marshes include Goldman (1918) on Wreck and Smith islands and Bailey (1946) on Wallops Island. Later, Paradiso and Handley (1965) found rice rats and meadow voles as the dominant marsh rodents in their survey of the small mammal fauna of the northernmost barrier island, Assateague Island. But the most complete survey of tidal marsh and island mammals was conducted in the mid-1970s by Dueser et al. (1979), who trapped on 11 islands; nine had marsh rice rats but only three islands also had meadow voles.

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All studies confirm the numerical dominance of marsh rice rats and, when present, meadow voles in the grass- and sedge-dominated tidal marshes.

Two studies used regular trapping on study grids to obtain density estimates of small mammals on barrier islands: Adkins (1980) in an Assateague Island marsh and Cranford and Maly (1990), on Wallops Island. Adkins (1980) found modest densities (10-15/ha) of both species in late autumn of two years with few or none of either species during the summer months. Cranford and Maly (1990) reported densities of 25 and 30/ha in two Novembers for rice rats but higher peaks in late winter (45 and 50/ha in March) for meadow voles. Later, Bloch and Rose (2005), also using capture-mark-recapture (CMR) methods on two study grids, report density estimates for both species in mainland tidal marshes in Northampton County, Virginia, with populations of both species fluctuating around a mean of 10/ha on one grid but on the second grid meadow voles had August-September peaks of 65 and 75/ha and rice rats had comparable densities but with peaks 3-4 months later. This paper describes the dynamics of populations of both species on the sites used by Bloch and Rose (2005) immediately after our study ended.

Meadow voles and marsh rice rats differ in two important ways: their diets and their activity periods. The meadow vole is considered a strict herbivore and the marsh rice rat an omnivore. Wolfe (1982) and others believe the marsh rice rat to be the second-most carnivorous North American rodent, behind the grasshopper mice (genus *Onychomys*) of the western US. However, a recent study of the diet of marsh rice rats in mainland marshes of Northampton County, Virginia indicates that local rice rat populations rely heavily on plant material throughout the year (Rose and McGurk, 2006), suggesting that Virginia populations may be more herbivorous than those living farther south (Negus et al., 1961; Wolfe, 1982). Regarding activity patterns, the marsh rice rat is strictly nocturnal, in part confirmed by being common in the diets of owls (e.g., Blem and Pagels 1973, Harris 1953, Jemison and Chabreck 1962), whereas meadow voles are intermittently active both day and night (e.g., Webster and Brooks 1981). Both species are similar in size, with fully adult meadow voles weighing 40-60 g and marsh rice rat adults slightly larger, up to 80 g.

The objectives of our study were to evaluate the population dynamics of the two species, including such features as density, survival rates, and reproduction, and to compare these patterns to those of other geographic populations. Our study lasted for a calendar year, from May 1994 through May 1995.

## MATERIALS AND METHODS

### The Study Sites

The two study sites were seaside marshes on Nature Conservancy property in Northampton County, Virginia. At Grid 1, located 4.4 km east of US Highway 13 at the southern edge of the village of Oyster, the vegetation was representative of the salt grass community. The dominant plants were *Spartina alterniflora*, *S. patens*, *Iva frutescens*, *Juncus roemarianus*, and on slightly higher ground, *Phragmites australis* (*communis*). Low-lying areas were subject to more frequent flooding, but most of the grid was on higher ground and remained relatively dry except during the high tides associated with the full moon. A thick ground cover of *S. patens* blanketed most of Grid 1. Grid 2 was located in a marsh locally known as Steelman's Landing, east of Townsend. A larger grid could be placed in this marsh, but the three rows closest to the

mud flats had sparse vegetation and deeper water during the periods of daily flooding. Grid 2 flooded less often than did Grid 1 because it was farther away from the shoreline. But the surface was flatter and so it flooded more uniformly, and was usually wetter than Grid 1 when flooding did occur.

#### Trapping Procedures

After preliminary trapping in March 1994 confirmed the presence of both species, grids were established at both sites, and monthly trapping began in May 1994. Grid 1 was irregular in shape to conform to the area of herbaceous vegetation; its 75 trapping stations were placed at 10-m intervals (maximum of 7 rows, 14 columns) for an effective trapping area of 0.75 ha. Grid 2 had 130 traps on a 13 X 10 grid, for a trapping area of 1.3 ha. One Fitch live trap (Rose, 1994) was placed at each grid coordinate (= station). However, because of the daily flooding or danger of flooding, traps were strapped onto floats made of 31 cm by 21 cm rectangles of 1.6 cm thick insulation Styrofoam, using large rubber bands. Wire ties and monofilament line secured each float to the wooden stake marking each coordinate on the grid. Thus, during periods of flooding the float raised the trap, enabling a rodent to swim to the trap or preventing trapped animals from drowning.

Each grid was trapped for three days per month, for a total of 1872 trap-nights on Grid 1 and 3584 trap-nights on Grid 2 (1 trap set for 1 night = 1 trap-night). Traps baited with mixed bird seed and sunflower seeds were set in the late afternoon and then checked early in the next three mornings. In summer, traps were locked open in the morning and set again in late afternoon to prevent death from confinement to a trap during the heat of the day. In winter, polyfill was added to each trap for insulation.

At first capture, each rodent was given a uniquely numbered ear tag, examined for its reproductive condition, weighed with a Pesola® scale, and released at the point of capture. Reproductive information included position of testes (abdominal or descended/scrotal) for males and for females we evaluated condition of the vagina (perforate or not), size of nipples (small, medium, large) and the condition of the pubic symphysis (closed, slightly open, open). Heavily pregnant females were recorded as such. We defined the age classes of meadow voles using the criteria of Krebs et al. (1969), with juveniles (<22g), sub-adults (22-29g), and adults ( $\geq 30$  g). For rice rats, we used the criteria of Wolfe (1985), with juveniles ( $\leq 30$ g), sub-adults (31-50g), and adults ( $> 50$  g). The trapping methods followed the animal handling guidelines of the American Society of Mammalogists (most recent: Sikes, Gannon et al. 2011). This study was conducted before Old Dominion University had established an IACUC protocol for the field study of wild mammals.

#### Statistical analysis

We used the Minimum Number Alive (MNA) method to estimate density of the populations (Krebs, 1966) and the statistical package JOLLYAGE to calculate time-specific survival rates of both adults and young and recruitment (Pollock et al., 1990). These parameters were analyzed using a Model-II 3-factor analysis of variance (ANOVA), with the factors being grid, season, and species. Where necessary, a Ryan-Einot-Gabriel-Welsch (REGWF) test was used to investigate the role of significant factors. Seasons were defined as summer (June-August), autumn (September-November), winter (December-February), and spring (March-May). Chi-Square tests

TABLE 1. Numbers of tagged *Oryzomys palustris* and *Microtus pennsylvanicus* and the other members of the small mammal communities at two locations in tidal marshes of the Atlantic coast in eastern Virginia. The numbers in parentheses are the number of captures for each sex and "unk" means the sex is unknown.

	Grid 1			Grid 2		
	Males	Females	Unk	Males	Females	Unk
<i>Oryzomys palustris</i>	30 (40)	28 (40)	3	118 (173)	72 (105)	28
<i>Microtus pennsylvanicus</i>	34 (35)	24 (29)	4	94 (115)	67 (83)	19
<i>Peromyscus leucopus</i>	6 (11)	6 (9)	4	2 (2)	1 (1)	0
<i>Mus musculus</i>	6 (7)	0	0	2 (2)	0	0
<i>Blarina brevicauda</i>	0	1 (1)	0	6 (11)	1 (1)	5

were used to determine whether sex ratios were unity. The  $p \leq 0.05$  level of significance was used for all statistical tests.

## RESULTS

### The Small Mammal Community

During the 13-month study period, 185 small mammals of five species were identified on Grid 1 (Table 1), of which 65 were rice rats and 62 were meadow voles; together these species constituted 84.7% of mammals. The other small mammals were white-footed mice (*Peromyscus leucopus*), house mouse (*Mus musculus*), and short-tailed shrew (*Blarina brevicauda*). Grid 2 yielded 218 rice rats and 180 meadow voles, comprising 96.8% of small mammals, and many fewer of the same three minor species (Table 1). Thus, rice rats and meadow voles numerically dominated both communities of small mammals.

### Population Density

The MNA density of *Oryzomys palustris* each month ranged from < 5/ha to 23/ha on Grid 1 (Figure 1a), showing no seasonal pattern, whereas on Grid 2 the population density rose steadily from September into winter, reaching a peak of nearly 45/ha in January (Figure 1b). Monthly changes in population density were similar for *Microtus pennsylvanicus*, i.e., mostly low densities and no seasonal pattern on Grid 1 but with highest density (30/ha) being attained in April on Grid 2.



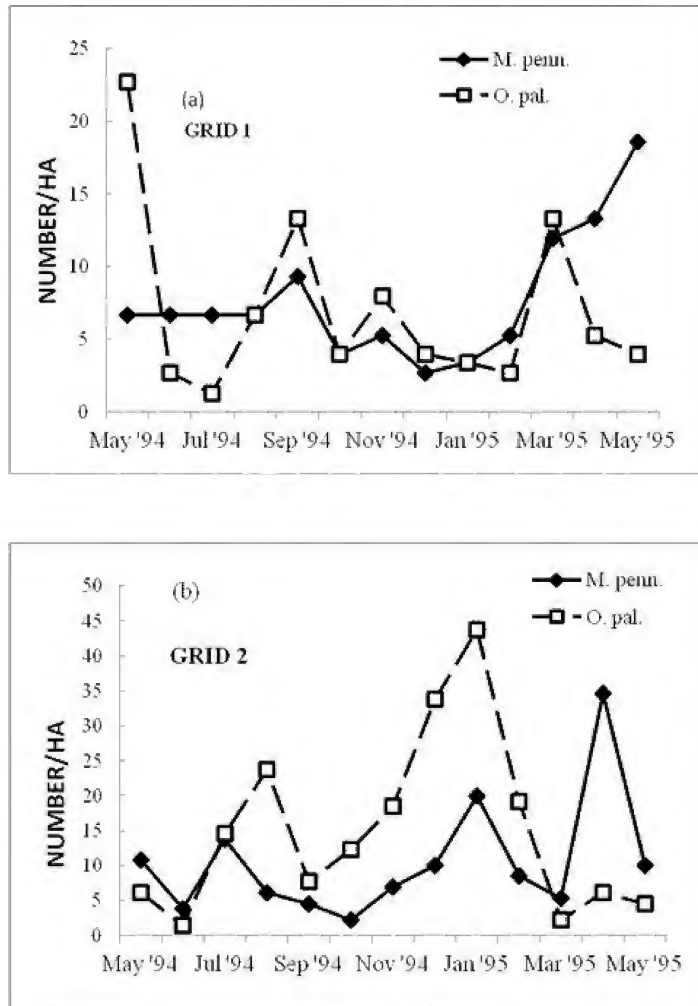


FIGURE 1. Monthly density changes for *Microtus pennsylvanicus* and *Oryzomys palustris* on Grids 1 (a, Oyster) and 2 (b, Steelman's Landing) in two coastal marshes in eastern Virginia.

### Survival Rates

Time-specific survival rates were calculated for juveniles and adults of both species. Grid ( $F=17.01$ ,  $df=1$ ), species ( $F=2.94$ ,  $df=1$ ), and the interaction of grid with species ( $F=39.62$ ,  $df=1$ ) all had significant ( $P < 0.05$ ) effects on adult survival. Mean monthly survival rate of adult meadow voles (0.524) was significantly ( $P < 0.05$ ) greater than that of rice rats (0.397). Summer survival rates (0.535 per month) were significantly higher than winter rates (0.372) but no other seasons differed. The interaction of grid and species also showed levels of significance in monthly survival rates (Grid 1: 0.753 for voles, 0.351 for rice rats; Grid 2: 0.316 and 0.438, respectively). Thus, survival rates of adult voles were much higher on Grid 1 than on Grid 2, whereas those of rice rats were more similar between grids. Except for meadow voles on Grid 1, the mean monthly survival rate was substantially less than 50 percent per month.

Juvenile survival rates could not be calculated for either species on Grid 1 because of small sample sizes. On Grid 2, the mean survival rate was 0.164 per month for juvenile *Oryzomys palustris* and 0.309 for juvenile meadow voles.

### Recruitment and Age Structure

The age structure of the *Oryzomys* population seemingly differed between grids, with juveniles being present on Grid 1 in May 1994, September, November, and March and April, whereas on Grid 2 juveniles were recorded in May 1994, July, August, September, November and December. No clear time of recruitment of young is evident, except that the absence of juveniles in January and February suggests no breeding in winter. For meadow voles, juveniles were observed on Grid 1 in May 1994 and April and May 1995, but on Grid 2 small numbers of juveniles were present in July, December, January, February, April and May 1995. Sub-adults dominated the age structure of both species during most months for populations on both grids.

### Sex Ratios

Although males of both species predominated on both grids (Table 1), significantly more males were observed only on Grid 2, the grid with higher densities of both species. The deviation from unity was greater for *Oryzomys palustris* (118:72,  $p < 0.001$ ) than for *Microtus pennsylvanicus* (94:67,  $p < 0.05$ ).

### Reproduction

Monthly changes in reproduction were analyzed using the position of the testes in males and nipple size in females as estimators of reproductive activity. Descended (scrotal) testes is a good (87-94%) predictor of reproductive status in male small mammals, but the best estimator for females of several rodent species is medium-large nipple size, with much less predictability at 72% (McCravy and Rose, 1992).

For meadow voles, males had peaks of breeding activity from late summer through autumn on both grids, with all males having descended testes during this period. Low rates were observed in early summer and mid-winter for male voles. Females showed a similar pattern, with low rates in early summer, increasing through autumn and peaking in October and November. June, with 0% with medium-to-large nipples, was the month of least breeding activity in female meadow voles.

Male rice rats showed greatest breeding activity in May, June, and November (100%) and lowest levels in late summer and late winter. Female reproductive activity

in rice rats was low in late summer and in winter, and highest in April, May, and November, with lows later in summer and in winter, suggesting a bimodal pattern of breeding for the species in eastern Virginia.

#### DISCUSSION

The composition of the small mammal communities in the two tidal marshes was identical. Rice rats and meadow voles predominated across the grids. Bloch and Rose (2005) later found these same five species, plus one mole at Grid 1; they also recorded nearly equal proportions of rice rats and meadow voles on each grid. Harris (1953) also found these two species as co-dominants in tidal marshes of the Chesapeake Bay in Maryland. These three studies on the mid-Atlantic coast represent the examples of these two rodents coexisting in mainland marshes where their distributions overlap.

The highest densities of marsh rice rats ranged up to 23/ha on Grid 1 and 45/ha on Grid 2 (Figure 1), whereas those of meadow voles peaked somewhat lower at 18/ha and 25/ha, respectively. Densities on Grid 1 were uniformly lower than on Grid 2, being greater than 10/ha only for 3 months by both species. By contrast, densities of 10 or more per ha were observed in half of the months on Grid 2: 7 times for rice rats and 6 times for meadow voles. Later, Bloch and Rose (2005) observed the same pattern of lower densities of both species on Grid 1 than on Grid 2. At Grid 1, they report densities for rice rats mostly in the 5-15/ha range, whereas densities of meadow voles were twice those values, with the high of about 40/ha in June 1995 resulting from the continued upswing in numbers seen earlier that spring. Bloch and Rose (2005, Fig. 1) recorded much higher densities of both species on Grid 2, with rice rats showing peaks of more than 60 and 80/ha in the two autumns. By contrast, high meadow vole densities, mostly in the 40-60/ha range, were less related to season. The densities observed by Adkins (1980) were similar to those on our Grid 1 for both species but Cranford and Maly (1990), also working on Assateague Island, reported higher densities of both species compared to our results on Grid 2. Thus, it seems likely that densities of both species vary in similar ways from year to year in both mainland and island marshes.

Survival can only be calculated for animals caught the next or a succeeding month, because only recaptured animals are known to have survived an interval of time. Animals caught in only one month (=transients), although they can be counted in estimates of density, contribute no information on survival. In our study, survival rates were highly variable, differing between grids and species. The survival rates of meadow voles on Grid 1 were twice those observed on Grid 2, whereas rates of rice rats were more similar between grids but low. Many studies (e.g., Krebs et al. 1969; Green and Rose 2009) have measured survival rates of small mammals, especially of voles and cotton rats; monthly survival rates are mostly in the 60-70% range. For our study, except for meadow voles on Grid 1, the other survival rates were 0.32-0.44, much lower than reported in studies conducted in upland habitats. Whether the low survival is attributable to mortality or migration is moot, but the dynamic nature of the tidal environment likely was an important factor too. Neither Bloch and Rose (2005) nor Harris (1953) reported survival rates.

The proportion of juvenile rice rats reached 50 percent of captures during the April-July period, especially in May of both years, but juveniles were absent in January and February. By contrast, juvenile meadow voles comprised about 25% of captures during

the March-May period on Grid 1 but on Grid 2 only once exceeded 10 percent of captures. Juvenile meadow voles were absent from both grids from August through November, suggesting that breeding occurs mainly during the winter and spring months. Populations of both species were dominated by sub-adults during many months, especially summer months. Adults often were missing from monthly estimates of age structure; only meadow voles on Grid 2 had adults present every month, and usually at 20-30 percent levels. One explanation for the relative lack of adult rice rats is that the 50-g criterion for adult weight is set too high for Virginia animals.

More males than females of both species were present on both grids, but significance was observed only on Grid 2. Bloch and Rose (2005) also found significantly more male rice rats than females on Grid 2 but not on Grid 1. Sex ratios for meadow voles were unity on both grids, a common finding across many geographic populations of this species (Reich 1981; Rose and Birney 1985). Harris (1953) reports catching significantly more male than female rice rats but equal numbers of each sex of meadow vole, results that conform to ours.

Reproduction in wild-caught rice rats is more difficult to assess than for many kinds of rodents, due in part to the dense fur in the inguinal region of both sexes and also to apparent changes in behavior in heavily pregnant females, which rarely enter live traps (RKR, personal observations). Rose and Dreelin (2011) report a relatively poor correspondence between external features of reproduction and the true reproductive condition of the rice rat as revealed at necropsy. For example, six of the 16 females found to be pregnant during necropsy had no external sign of pregnancy: no perforate vagina, small nipples, and no separation of pubic bones. Rose and Dreelin (2011) learned, using logistic regression, that perforate vagina was the best predictor of breeding status for female rice rats and descended testes for males. Large body mass also was useful in predicting breeding status in rice rats. Using these external indicators, rice rats bred mostly in spring and autumn in our study, with a lull in mid-summer and no breeding in winter, a pattern similar to that observed in a Delaware marsh by Edmonds and Stetson (1993). The timing of the entry of juveniles into the trappable population also supports this assessment.

By contrast, the highest proportion of reproductively ready male meadow voles was observed from late summer through autumn, with lower indices in the other months, especially in summer. Female voles had high reproductive indices in spring and autumn, and lowest values in summer. Thus, a mid-summer breeding lull was apparent in both sexes of meadow vole. Interestingly, no juvenile meadow voles were trapped from late summer through autumn, suggesting that their rates of survival were poor then.

In conclusion, marsh rice rats and meadow voles are the dominant small mammals in mainland marshes in eastern Virginia, both species having made accommodations to the daily flooding cycle. Rice rats generally had higher densities than meadow voles, both species had breeding peaks in spring and autumn with less breeding in summer and especially in winter, and sex ratios favored male rice rats but were unity in meadow voles.

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## First Record of Pond Sliders (*Trachemys scripta scripta* and *T. s. elegans*) at Fredericksburg, Virginia with Observations on Population Size, Age and Growth

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### ABSTRACT

We conducted a turtle mark-recapture program within a 160 m stretch of the Fredericksburg Canal with standard, baited hoop nets from May to July 2012 to determine if a population of the introduced Red-eared Slider (*Trachemys scripta elegans*) and Yellow-bellied Slider (*Trachemys s. scripta*) are established in this area. We captured and marked nine Red-eared Sliders (1 male, 8 females) and estimated a population size of 23 individuals. Most were reproductively mature. The established population in the canal may be a source of introduction into the Rappahannock River.

Key Words: Red-eared slider, *Trachemys scripta elegans*, distribution, age, introduced

### INTRODUCTION

Pond sliders (*Trachemys scripta*) are found throughout the southeastern and middle United States, ranging from the southeastern coastal plain west to Kansas, Oklahoma, and New Mexico (Ernst and Lovich 2009). However, they are not native to areas outside of southeastern and southwestern Virginia (Buhlman et al. 2008). Three subspecies are recognized; the Yellow-bellied Slider (*T. s. scripta*), the Red-eared Slider (*T. s. elegans*), and the Cumberland Slider (*T. s. troosti*). As a result of their popularity in the pet trade pond sliders have been introduced in all 48 states and Hawaii and breeding colonies are established on all continents except Antarctica (Ernst and Lovich 2009). The Red-eared Slider is not native to Virginia (Mitchell 1994; Ernst and Lovich 2009). Both Yellow-bellied Slider and Red-eared Slider have been introduced in Maryland and Northern Virginia, where subspecific intergradation readily occurs (Iverson 1992; Ernst et al. 1997). Specifically, populations of both subspecies are established in Mason Neck Wildlife Refuge of Fairfax County (Mitchell 1994). Mitchell (1994) has recorded Red-eared Sliders in Hanover County and other southeastern counties and municipalities where they sometimes intergrade with native Yellow-bellied Sliders. Another population of red-eared sliders occurs in Henry County, south-central Virginia (Mitchell, 1994).

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Yellow-bellied Sliders are native to southeastern Virginia and their range extends throughout the Coastal Plain of the Carolinas, Georgia, northern Florida and the eastern portion of Alabama (Mount 1975; Stevenson 1976; Ashton and Ashton 1991; Gibbons and Semlitsch 1991; Mitchell 1994; Palmer and Braswell 1995; Conant and Collins 1998; Bartlett and Bartlett 2006; Beane et al. 2010). The Red-eared Slider's native range extends from the midwestern states as far east as West Virginia, west into eastern New Mexico, and south into northeastern Mexico (Green and Pauley 1987; Conant and Collins 1998; Bartlett and Bartlett 2006; Phillips et al. 1999; Dixon 2000). The two subspecies naturally intergrade throughout much of Alabama resulting in an admixture of parental forms and intermediate patterns (Mount 1975).

In April 2011 a specimen of the Yellow-bellied Slider was captured in the canal in the City of Fredericksburg by a student and brought to the senior author for identification. The individual appeared to be an immature female about 130 mm plastron length. The specimen was taken by dip net about 300 m from the canal's downstream confluence with the Rappahannock River. The canal is about 3.2 km in length. This is the only reported occurrence of this subspecies in the vicinity of Fredericksburg, Virginia and the Rappahannock River drainage. This individual was returned to the canal after being photographed. The northernmost native occurrence of Yellow-bellied Sliders is in Hanover County in the headwaters of the Chickahominy River (Kleopfer and Niccoli 2010). Given these observations we sampled turtles in the Fredericksburg Canal to determine if a population of Yellow-bellied Sliders is established.

#### METHODS

From May 30 until July 19, 2012 we collected turtles in the Fredericksburg Canal with commercial, single throat hoop nets (3 ft. diameter, 1 in. mesh) available from Memphis Net & Twine Co., Inc. Each was baited with sardines and set for 24 h period. Combinations of four and eight nets were set and checked the following morning. All were set in an area covering about 160 m of the canal in the vicinity where the original Yellow-bellied Slider was captured. Upon capture turtles were weighed ( $\pm 10$  g) with Pesola® scales, plastron length measured ( $\pm 1$  mm), and given an identifying code by filing a combination of notches into the marginal scutes (Figure 1) (Mitchell 1988). Some individuals were aged by counting annuli on the plastron (Buhlmann et al. 2008). We used a Jolly-Seber model (POPAN formulation) available as a submodule of the program MARK (White and Burnham 1999) to estimate population size (available from USGS Patuxent Wildlife Research Center Software Archive).

#### RESULTS

We captured five species during 152 trap nights: Snapping turtle (*Chelydra serpentina*), Eastern Painted Turtle (*Chrysemys picta picta picta*), Northern Red-bellied Cooter (*Pseudemys rubriventris*), Eastern Musk Turtle (*Sternotherus odoratus*), and Red-eared Slider. No Yellow-bellied Sliders were captured.

Nine Red-eared Sliders (1 male; 8 female) were captured; three females were each captured twice. Time between first and second capture ranged from 3 to 27 days. Females ranged in size from 117-235 mm (Table 1). The single male measured at 115 mm. Both male and female turtles are considered reproductively mature based on the smallest known size at maturity as reported for populations of pond sliders in Georgia



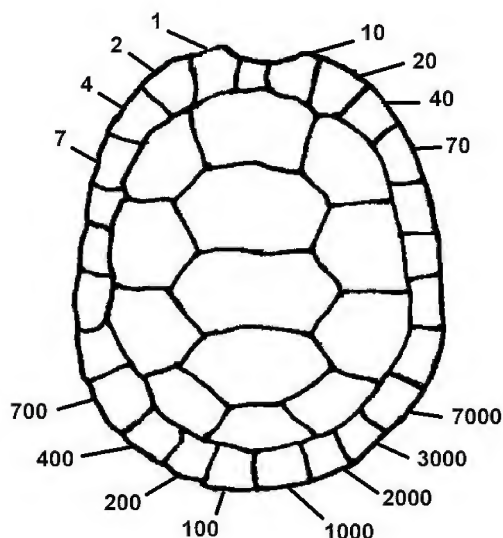


FIGURE 1. Number system for marking the carapace of turtles after Mitchell (1988). This system allows for identification of 9999 individuals.

by Gibbons and Greene (1990) and in Virginia by Mitchell and Pague (1990). Slider ages ranged between 3-6 years for females; the male was 4 years old. The oldest female (200 mm) we were able to age was not the largest. Due to a lack of annuli or numerous abrasions we were unable to age four of the females.

Program MARK estimated that 23 Red-eared Sliders occur within the sampling area (95% CI 10-114) which consisted of about 160 m of canal extending upstream from the lower end. Based on information from Schubauer et al. (1990) for mean length of home range we estimate a total population of 100 to 500 individuals within the entire length of the canal. Given the range of values, this population size estimate may not be reliable.

#### DISCUSSION

The absence of Yellow-bellied Slider suggests that there is no established population in the Fredericksburg Canal. The turtle captured in April 2011 is likely the only individual present. Based on photographs it appeared to be about 130 mm and likely a female.

TABLE 1. Age, as determined by counting annuli on the plastron, and size of Red-eared Slider (*Trachemys scripta elegans*) from the Fredericksburg Canal at Fredericksburg, Virginia, May - July 2012.

Age	Sex	Plastron length (mm)	Weight (g)
4	M	115	270
?	F	117	1100
3	F	160	730*
4	F	190	1325
5	F	221	1425
6	F	200	1350
?	F	225	1550
?	F	225	2250
?	F	235	2100

\* - immature individual based on size.

The occurrence of the Red-eared Slider is a new record for the Rappahannock River drainage (Figure 2). Due to the wide extent of introductions of this species its presence is not unexpected. However, because both male (albeit only one) and female individuals were captured there appears to be an established population. Presumably due, in part, to its extensive introduction throughout the world the International Union for Conservation of Nature has placed the Red-eared Slider on its list of the World's Worst Invasive Species (ICUN 2012).

Age determination for all individuals proved problematic due to abrasions or the lack of annuli. This was especially true for the larger individuals. The size of the largest individuals captured indicate that these were mature females (Table 1) because plastron lengths and weight correspond with that reported by Mitchell and Pague (1990) for female pond sliders from southeastern Virginia. However, there is a discrepancy in age as reported here. Slider females have been reported to reach maturity at eight years of age (Gibbons and Green, 1990; Mitchell and Pague, 1990) yet our oldest mature individual was 6 years. Wilson et al. (2003) noted that many of the studies they reviewed found this method of aging to be reliable for young adults only (*i.e.*, prior to reaching sexual maturity). This is consistent with our observations. Mitchell and Pague (1990) reported the a minimum plastron length of 204 mm at maturity for pond sliders in the Great Dismal Swamp National Wildlife Refuge, Suffolk, Virginia. Ages relative to size reported in this study differed from those expected based on reports for Yellow-

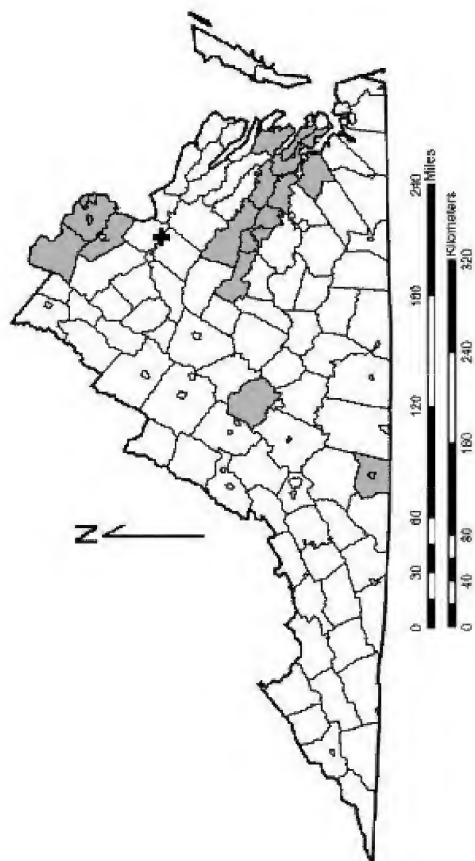


FIGURE 2. Shaded areas indicate known occurrences of the non-native Red-eared Slider (*Trachemys scripta elegans*) in Virginia as reported on the Virginia Department of Game & Inland Fisheries website (February 2012) (<http://www.dgif.virginia.gov/wildlife/information/?s=030077>). First report of population discovered in the Fredericksburg Canal (+), Rappahannock River drainage basin (May-July 2012).

bellied Sliders in Virginia (Mitchell and Pague 1990). However, the inaccuracy or inability to age large individuals is consistent with what is reported by Wilson et al. (2003).

Our results show that a Red-eared Slider population is established in the Fredericksburg Canal at Fredericksburg, Virginia. Given the ease with which this turtle adapts to areas where it where it this population may soon spread to the Rappahannock River and its adjoining bodies of water, if it has not already done so.

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**Proceedings of the 91<sup>st</sup> Annual Meeting  
Virginia Academy of Science  
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May 22-24, 2013**

**Aeronautical and Aerospace Sciences**

EVOLUTION OF U.S. MILITARY AIRCRAFT. M. Leroy Spearman & Robert W. Heath, Langley Research Center, Hampton, VA & Independent Contractor, Newport News, VA. The airplane was accepted as a part of the military system in August 1907 with the establishment of an Aeronautical Division of the U.S. Army Signal Corps. An airplane was procured from the Wright Brothers and flight training began at Fort Meyers, VA. In August 1909 an improved Wright Flyer was formally accepted and was identified as 'Airplane No. 1'. Another U.S. designer was becoming well known and in 1911 a Curtiss airplane was the second airplane to be sold to the U.S. Army. The first appropriation for military aeronautics was in the 1912 War Department budget for the sum of \$125,000 and orders were placed for three Wright airplanes and two Curtiss airplanes. European countries, on the brink of World War I, were making progress in the development of military aircraft. The U.S. entered the war in 1917 but had no military aircraft. U.S. volunteer pilots did gain experience while flying British and French aircraft and the U.S. industry gained experience by manufacturing some European airplanes. Following the war, the U.S. acquired a number of British and French airplanes for the Army Air Service. Native aircraft were soon to appear, primarily trainer airplanes and early Martin bombers. Concern that the U.S. was lagging in aeronautical research led to the establishment of the National Advisory Committee for Aeronautics (NACA). An aeronautical laboratory was constructed at Langley Field, VA and, since 1920, the laboratory has contributed to the technological growth of U.S. military aircraft as they evolved from the classic propeller-driven fabric-covered biplane to the all-metal monoplanes. In the 1940's jet-propulsion was introduced and the flight envelope was changed to include transonic and supersonic flight. More recently some military aircraft designs have employed stealth technology that is intended to make the aircraft less visible to most tracking systems – example is the F22 Raptor stationed at Langley Field, VA.

DELINEATION OF AERODYNAMIC PROBLEMS WITH WIND TUNNEL TESTS. M. Leroy Spearman, Langley Research Center, Hampton, VA. The use of wind tunnels in the development of new aircraft and missile concepts is well known. However the wind tunnel is also useful in establishing the characteristics of existing aircraft or missiles. As flight speeds increased compressibility of the air became a major problem. Regions of sonic flow caused some separated flow that reduced the control effectiveness. Wind tunnel tests indicated that blunting the trailing edge of flap-type controls to reduce the surface slope would eliminate the separated flow and restore

the control effectiveness. Blunted trailing edges. were applied to the ailerons and the horizontal tail of several high-speed aircraft and the roll control and pitch control was restored. Further research lead to the development of the all-moving tail that could be moved symmetrically for pitch control and differentially for roll control. In 1949 Convair produced the delta-winged F-102 supersonic aircraft that had difficulty in achieving the desired supersonic speeds. Production was halted and NACA-Langley undertook an investigation of the concept. A major revision involved the application of the transonic area rule that resulted in sliming the body in the region of the wing attachment- the coke bottle' contour. The transonic drag rise was reduced, supersonic speed was achieved and production was resumed. Wind tunnel tests in conjunction with analytical results can be useful in determining the performance characteristics of existing aircraft. One example is the Soviet MiG-25 airplane first seen in a 1967 airshow. The airplane was said to be capable of Mach number 3 flight. There was some doubt of this performance in the Western World. Estimates of the overall planform and profile shape could be gotten from airshow photographs. A study was undertaken at NASA-Langley to determine the cross-sectional shape that would be required to meet the speed. A wind tunnel model was built and tests verified the speed. In 1976 a MiG-25 defected to Japan and a detailed examination of the airplane indicated that the NASA-Langley model was essentially correct.

VARIATIONS OF VARIABLE GEOMETRY AIRCRAFT. M. Leroy Spearman, Langley Research Center, Hampton, VA. The geometric shape of an aircraft usually consists of a fuselage, wing and tails and a propulsion system. The fuselage provides the space required for the cargo, equipment and crew. The wing provides the lift required to sustain flight. The tails provide for stability and control. The propulsion system provides for forward movement of the aircraft. The shape and arrangement of these components are designed in such a way as to assure that the mission requirements for the aircraft are met. Thus for different mission requirements a different geometric shape is required. This lead to the concept of a single aircraft having variable geometry that would be suitable for multiple missions. An example of such an aircraft is the General Dynamics F-111 in which the wing panels can be set at a low sweep position for take-off and landing and low-speed flight – for high-speed flight the wing panels are rotated back to a high sweep angle. With the F-111 the entire wing panels are moved using inboard pivots. Some research has been done with outboard pivots in which only a portion of the wing panel is swept. And some research has been done with only a single pivot wherein the entire wing is rotated into what is known as a skewed wing. Several aircraft having variable sweep wings have been produced in the U.S., and in several European countries and in the Soviet Union.

### **Agriculture, Forestry, and Aquaculture**

DOES PROXIMITY TO COMPOST PILES AFFECT FLY POPULATIONS IN HIGH TUNNEL TOMATO? Mark Kraemer, Walter Mallory & Steven Pao, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. Compost is commonly used in organic vegetable production but it is not known if nearby compost piles pose a health threat. This study evaluated fly populations in two high tunnels,



with and without compost. Both tunnels had 3 rows of bush tomatoes. The treatment tunnel had tubs of manure and straw placed in each corner. Flies were monitored over a period of 10 weeks using 15 x 25 cm yellow sticky cards, one in each quadrant of each tunnel. The control tunnel was 5 m distant to the treatment tunnel. Four types of filth fly were found in the tunnels, plus hover flies and Tachinid parasitoids. The lesser house fly (*Fannia canicularis*) was the most numerous and increased over time. Other flies were the common house fly (*Musca domestica*), stable fly (*Stomoxys calcitrans*), and black garbage fly (*Hydrotaea aenescens*). There were no significant differences between filth fly populations between tunnels. Thus, a distance of greater than 5 m from compost piles is required to reduce filth fly populations.

EFFICACY TESTS OF VERNONIA LEAF EXTRACT AND FRACTIONS TO CONTROL SMALL RUMINANTS GASTROINTESTINAL PARASITES (GIN). F. Djibodé Favi, A. B. Yousuf, S. A. Wildeus, W. H. Mallory & M. E. Kraemer, Agriculture Research Station, Virginia State University, Petersburg VA 23806. Gastrointestinal nematodes (GIN), were the greatest danger to the small ruminant industry worldwide and *Haemonchus contortus* (Rudolphi) Cobb is one of the most pathogenic to ruminants. It piercing the abomasum causes a number of significant complications that can lead to death. Leave from vernonia varieties were used for thousand years in the old world to cure GIN in animal and human. Tested extracts were mostly inconclusive in 3600 documented records. The objective was to test fractions of leaf extract to determine efficacy conditions of *Vernonia galamensis* (Gilbert) from Africa. Aqueous and oily fractions from leaf extract were parted to obtain concentrate fractions. Fractions were applied to larva in 12- cell plate (10 larva/ $\mu$ l) and larval mortality was assessed for three days. The controls were 30% solvent and commercial dewormers Cydectin. Eggs filtered from feces of infested animal were diluted to 50 eggs/100 $\mu$ l were tested in 12-cell plate. Hatching were monitored from 1-3 days. Cydectin kills L3 larva within 1-3 days post treatment (dose dependent). Egg leaks and the shell has a round hole. Fraction A kill larva between 1-2 days post treatment. Larva vomit struggle and die. Egg shows atypical frozen yolk. Fraction A was selected for further studies.

TEMPERATURE EXTREMES OF THE BROWN MARMORATED STINK BUG (*Halyomorpha halys*). John D. Aigner, Thomas P. Kuhar, Ashley K. Lohr, & Christopher R. Philips, Virginia Tech, Department of Entomology, 216 Price Hall, Blacksburg, VA 24061. Today's global economy continues to grow, increasing the chances that invasive species become established in the United States. Brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a major agricultural pest that was accidentally introduced in Pennsylvania and is rapidly spreading throughout the North America. This project was designed to better understand the bug's physiology and its ability to withstand a range of temperatures so that we can better understand the potential climatic and geographic limitations of the insect. This was done by monitoring the lethal high temperature of BMSB and by identifying the supercooling points (SCP's) associated with BMSB at several intervals throughout the year. Field-collected and lab-reared BMSB egg masses, nymphs, and adults were exposed to temperatures ranging from 35 to 45°C (95 to 113°F) for four hours or until all bugs died, whichever was shorter. Temperatures above 41°C (104°F) resulted in

significant mortality of all stages of BMSB. All life stages of BMSB survived exposures to temperatures below 40°C (104°F). Supercooling points were determined to range from -8°C (17.6°F) to -22°C (-7.6°F) using field collected BMSB. These SCP's change throughout the year however, no discernible pattern was recognized. These SCP's have been monitored from December of 2011 through April 2013.

OMNIVORY AND PREDATOR FUNCTION IN AGRICULTURAL ECOSYSTEMS. C. R. Philips, T. P. Kuhar & D. A. Herbert, Dept. of Entomology, Virginia Tech, Blacksburg, VA 24061. Understanding how buckwheat (*Fagopyrum esculentum* Moench) companion plantings impact natural enemy abundance, and pest suppression in adjacent crops, may provide alternative control methods thereby slowing the rate of insect resistance to pesticides and reducing pesticide exposure to the applicator and surrounding habitats. Buckwheat has been widely used as a companion planting on vegetable farms, vineyards, and orchards to supply nectar and pollen and encourage arthropod natural enemy populations. However, scientific data demonstrating the true biological control benefit of such companion plantings are scarce. The primary objective of this project was to determine the impact of buckwheat companion planting on lepidopteran pest abundance, parasitoids abundance, parasitism, and the predator and parasitoid communities in collards. Overall abundance of pest caterpillars did not vary with distance and imported cabbageworm was the most abundant species. Parasitism of imported cabbageworm larvae did not differ with distance from available nectar sources. Abundance of parasitoids was not significantly different with distance, and the average rate of parasitism was 50%. Even at low numbers, it is likely that parasitoids play an important role in lepidopteran population dynamics. It also appears, however, that generalist predators may also be playing an important role. Future research will investigate the biology, habitat use, and predation impacts of some of these predators.

MONOSEX CULTURE OF FRESHWATER SHRIMP (*Macrobrachium rosenbergii*). Brian Nerrie, Virginia Cooperative Extension, Virginia State Univ., Petersburg VA 23806. In Virginia sustainable production of freshwater shrimp (*Macrobrachium rosenbergii*) has been shown to be an excellent agriculture diversification in the tobacco growing region, especially for limited-size farms. Research and demonstrated farming success in Israel, India and Bangladesh indicated improved yields for all-male shrimp populations. Research in Mississippi and Kentucky confirmed higher yields. Benefits and disadvantages of all-male or all-female monosex populations are discussed. Adoption of all-male production practices in Virginia would result in enhanced per acre production levels and larger individual animal size. However, multiple factors need to be considered by the private sector. The factors impacting on profits are: availability of all-male juveniles, stress from time and distance to stocking, survival rate, higher juvenile costs and whether consumer preference for higher prices for larger shrimp exceeds a larger quantity of smaller shrimp of the same value. Profitability will be the major determinant if monosex culture is adopted.

DISEASES OF CHANNEL CATFISH REARED IN CAGES IN VIRGINIA. David Crosby, Virginia Cooperative Extension, Virginia State University, PO Box 9081, Petersburg, VA 23806. The main production method for growing catfish in Virginia

is using cages in farm ponds. Most catfish fingerlings used for stocking cages comes from out of state commercial farms. After the initial stocking, parasitic and bacterial outbreaks can occur up to three days later. *Aeromonas hydrophila* and *Flavobacterium columnare* are the most common bacterial pathogens that cage producers will encounter. These pathogens are quite common in the aquatic environment and are the leading causes of disease problems in cages. Columnaris infections can occur within one day after stocking catfish into cages. A highly pathogenic strain of *Aeromonas hydrophila* that originated in Alabama has been diagnosed from catfish stocked in cages in Virginia. A study conducted at Virginia State University (VSU) found that catfish fingerlings imported from commercial farms usually had external parasites. *Trichodina* was found on catfish from four of the six commercial catfish fingerling shipments to VSU from 2007 – 2009. All shipments had *Henneguya*, the causative agent of proliferative gill disease. *Trichodina* is the most common parasite on catfish in cages that causes mortalities. In February of 2013, winter saprolegniasis or winter kill occurred in a caged catfish operation resulting in the high mortalities. This incident occurred in a pond with very low alkalinity, less than 17 ppm. Anytime a fish farmer is stocking catfish into cages from a commercial fish farm may increase the potential risk of a disease outbreak.

CAN STANDARD SOIL TESTS PREDICT ORGANIC BLUEBERRY PRODUCTION IN PLOTS WITH DIFFERENT SOIL AMENDMENT TREATMENTS? Roman J. Miller, Dept. of Biol. Eastern Mennonite University, Harrisonburg, Virginia 22802. What best predicts blueberry productivity: Soil tests? Foliar analysis? Plant growth? This question was examined in four blueberry plots with different mulches incorporated into their soil: organic horse manure (OHM), organic sheep manure (OSM), organic pine straw (OPS), and organic Planter Choice (OPC). Soil tests revealed normal or high nutrient levels in all plots. pH values were near optimal in OHM and OPC, but too high in OSM and too low in OPS. Soil respiration, foliar nutrient and chlorophyll values were within expected ranges or optimal in all plots. Growth parameters such as bushiness for Duke was greatest in OPS; while Bluecrop bushiness was greatest in OSM. Average plant height for OPS and OPC bushes was 50% greater than OSM bushes and 100% greater than OHM bushes. Berry productivity in the OPS and OPC plots doubled the OSM plot and exceeded the OHM plot threefold! While soil profiles and foliar analysis both failed to adequately predict berry productivity, simple bush height in unpruned young blueberry bushes emerged as the single best predictor of blueberry productivity. (Supported by USDA Specialty Crop Grant #2011-546)

#### Posters

ANALYSIS OF ANTHOCYANINS AND TOTAL PHENOLIC COMPOUNDS IN BLUEBERRIES: METHOD DEVELOPMENT AND PRELIMINARY RESULTS. A. Kniss, E. Harnish, S. Cessna & R. Miller, Departments of Biology and Chemistry, Eastern Mennonite University, Harrisonburg, VA 22802. Anthocyanidins and other phenolic compounds are known to have antioxidant capacity. Blueberries have been reported to be one of the richest sources of antioxidants among common fresh fruits. Interest in the micronutrients of blueberries has grown with increasing evidence of

possible health benefits. In order to compare the antioxidant profile of different varieties of high bush blueberries growing in differing conditions, several methodologies were tested. We found that two different UV-Visible spectroscopy-based measures of antioxidants were manageable for testing large numbers of berries. These are the FRAP assay (ferric ion reducing antioxidant power) and the Folin-Ciocalteu assay of total phenolic compounds. Our initial findings with these two assays indicate that Duke and Jersey varieties are lower in antioxidants than Blue Crop and Blue Gold. Several methods for HPLC quantification of acid hydrolyzed anthocyanidins were also tested; here we report the most successful extraction, hydrolysis and chromatography method. These three measures provide quantitative analysis of antioxidants in blueberry cultivars. Each method was developed to the point of functional use, and will be used to determine health of organically grown blueberries and compare them to conventionally grown blueberries. (Supported by USDA Specialty Crop Grant #2011-546)

ABUNDANCE OF APPETITE REGULATORY FACTOR mRNA IS INFLUENCED BY 3-HOUR FEED WITHDRAWAL IN CHICKENS SELECTED FOR HIGH AND LOW BODY WEIGHT. W. Zhang, L. A. Nelson, P. B. Siegel, M. A. Cline, & E. R. Gilbert, Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061. Long-term (55+ generations) selection for low (LWS) and high (HWS) body weight in chickens has resulted in a 10-fold difference in body weight at selection age and correlated responses in appetite and body composition. Some of the LWS are anorexic, while HWS are hyperphagic and obese. We hypothesized that differences in appetite regulatory factor mRNA between the lines would be accentuated by food withdrawal. Five-day old male LWS and HWS chicks (n=24) were randomly divided into 2 treatments; 1) continuous access to a starter diet or 2) 3 h feed withdrawal, after which hypothalamus was collected. The mRNA abundance of *AGRP*, *NPY*, *NPYR1*, *MC4*, *GLUT1*, and *FOXO1* was measured by real time PCR. Data were analyzed by ANOVA using the Glimmix procedure (SAS 9.3). For *AGRP*, *NPY* and *NPYR1*, there was greater ( $P < 0.05$ ) abundance in LWS than HWS, whereas for *FOXO1* and *MC4* there was greater ( $P < 0.05$ ) expression in HWS. Food withdrawal affected hypothalamic *NPY* mRNA abundance, where *NPY* mRNA was greater in fed LWS chicks as compared with fed HWS ( $P < 0.05$ ) and fasted LWS chicks ( $P < 0.05$ ). In conclusion, there was greater mRNA abundance of food intake-stimulatory factors in LWS and greater abundance of satiety-inducing factors in HWS. These results suggest that differences in food intake between LWS and HWS are associated with differences in the appetite circuitry of the hypothalamus and that these differences are accentuated under certain feeding conditions.

### Astronomy, Mathematics, and Physics

AN INEXPENSIVE RADIO TELESCOPE IN A COLLEGE PHYSICS LAB, AN UPDATE. C. Crook & T.C. Mosca III, Dept. of Chem. and Phys., & Dept. of Math, Rappahannock Community College, Warsaw VA 22572. An amateur radiotelescope on the Glens campus of Rappahannock Community College was used by first-year physics students to conduct original research on solar radio frequency emissions. Most

notably, RF signals were recorded that fit the characteristic profile of solar flare emissions that approximately coincided with a flare confirmed by NASA and other radio observatories. Slight anomalies in the time that this flare was recorded were discussed but not resolved. The project also provided a logical context for the application of physics theories that are not directly observable, such as energy transmission by magnetohydrodynamic waves through the solar plasma.

FLUCTUATIONS IN A THERMOELECTRIC AND INFRARED POWER GENERATION. H. S. Mann<sup>1</sup>, A. E. Masters<sup>2</sup> & G. X. Scarel<sup>1</sup>, <sup>1</sup>James Madison University, Dept. of Physics and Astronomy, Harrisonburg, VA 22807, & <sup>2</sup>Custom Thermoelectric, Bishopville, Maryland 21813. In recent years, the interest in alternative energies has risen greatly. Infrared radiation represents a significant portion of the solar spectrum which is worth exploiting. Capturing infrared radiation and converting it into usable electricity has been demonstrated using power generators. However there is evidence that power generators interact differently with heat or infrared radiation. This work addresses the fluctuations in voltage observed in a power generator interacting with heat in an open and isolated environment. The results are then compared with the fluctuations in voltage generated by infrared radiation on a power generator in an open and isolated environment. The findings from these measurements contribute to further understanding the distinction between infrared and thermoelectric power generation, as well as the fundamental physics of the power generators involved.

A WISE SEARCH FOR EXCESS MID-INFRARED EMISSION FROM 100,000 NEARBY M DWARFS IN THE SUPERBLINK PROPER MOTION SURVEY. C. A. O'Donnell<sup>1,2</sup>, S. Lepine<sup>2,3</sup> & B. Rojas-Ayala<sup>2</sup>, <sup>1</sup>Department of Astronomy, University of Virginia, Charlottesville VA 22904, <sup>2</sup>American Museum of Natural History, New York NY 10024, & <sup>3</sup>City University of New York, New York NY 10075. We searched the Wide-field Infrared Survey Explorer (WISE) catalog to identify mid-infrared counterparts of 100,000 M dwarfs within 100 parsecs of the Sun selected from the SUPERBLINK proper motion survey. An M dwarf with infrared excess can be a sign of dust or planetary material that is re-emitting absorbed optical light; both possibilities would presumably increase a system's overall infrared flux compared to the expected flux of the star alone. Additionally, such systems are likely to be very young. The WISE survey covered 4 bands centered at 3.4, 4.6, 12, and 22 micrometers (W1, W2, W3, and W4 respectively). Since almost all of the M dwarfs did not have reliable W4 magnitudes, we focused on the W1-W3 color and found that the vast majority of M dwarfs had  $W1-W3 < 0.5$ . After systematic examination of all stars with redder colors (i.e.,  $W1-W3 > 0.5$ ), including visual verification of the WISE images, we have identified 172 M dwarfs with clear infrared excesses ( $0.5 < W1-W3 < 2.48$ ) and 223 additional candidates with possible but weaker infrared excesses ( $0.5 < W1-W3 < 1.27$ ). All these systems, especially the 172 high-excess systems, should be high priority targets for follow-up observations, including exoplanet survey programs. Finally, we identified 5 stars with particularly high infrared excesses ( $1.0 < W1-W3 < 2.48$ ), and their spectral energy distributions were consistent with a dust model. Future observations will confirm the source of the excess emission.

THE SIGNATURE OF RADIATIVE POLARITONS: DIELECTRIC FUNCTION AND SIMULATED SPECTRA. Yosyp Schwab & Giovanna Scarel, James Madison University, Harrisonburg, VA 22807. Surface phonon polaritons have recently received a lot of attention for their role in heat transfer in nano-structured materials. However, much less attention has been given to radiative polaritons and their vital role in determining the optical properties of insulating oxides and in infrared (IR) power generation. Here it is shown through experiment and simulation that radiative polaritons radiate in the far-IR/microwave frequency region. In order to obtain the simulated results, the classic dielectric function for insulating oxides was modified. A contribution including the radiative polaritons was added, leading to an excellent agreement with the experimental spectra. This is the first time that the location of the frequency range of radiative fields has been experimentally shown, as well as the existence of the radiative fields themselves.

INSTRUCTIONAL INSTRUMENTATION LAB EXPERIMENTS AT JAMES MADISON UNIVERSITY. Joseph D. Rudmin, & Anne Henriksen, Dept. of Integrated Science and Technology, James Madison Univ., Harrisonburg, VA 22807. James Madison University college students experience a series of laboratory activities illustrating critical concerns in designing laboratory experiments, and gain experience with common representative measurement instruments. The laboratory experiences maximize clarity of illustration of concepts, within a very modest laboratory budget. The laboratory experiences build on each other. National Instruments compact DAQ hardware and LabView software are used for the most rapid data acquisition that students collect.

MATTER-ANTIMATTER ASYMMETRY AND NEW STATES OF MATTER WITH THE BELLE EXPERIMENT. Leo Piilonen, Dept. of Physics, Virginia Polytechnic Inst. and State Univ., Blacksburg VA 24061-0435. I introduce the broad topic of elementary particle physics and how it helps us to understand the nature and workings of the universe at its most fundamental level. I then illustrate this topic through the studies of the difference in behavior between matter and antimatter and the search for new states of matter as probed in the Belle experiment and its successor, Belle II, operating at the High Energy Accelerator Research Organization (KEK) in Japan.

NUMERICAL MODELING OF THE CLIMATE CHANGE IMPACT ON RARE GENOTYPES IN NATURE. Charles L. Armstrong, Iordanka N. Panayotova, & Lisa Horth, Dept. of Mathematics & Statistics & Dept. of Biological Sciences, Old Dominion Univ., Norfolk, VA 23529. This study uses computer models created using MATLAB software to analyze the effects of climate change on rare genotypes that are activated by temperature-sensitive enzymes. The primary focus of this study is the effect that these changes would have on the black-spotted mosquitofish (*Gambusia holbrooki*). We also analyze the effects that these temperature increases and resulting decreases in melanic mosquitofish inheritance will have on the overall mosquitofish population. For this study we used a model previously published by Dr. Panayotova simulates the life cycle of this fish. For this study we have created models that account for rising temperatures, yearly temperature fluctuations, the relationship between rising temperatures and melanic inheritance, and different patterns of temperature increases.

Our results indicate that increasing temperatures will be devastating for the melanic mosquitofish population, resulting in extinction within 1000 years. Despite these findings, our simulations also indicate that if the temperature stabilizes before the melanic mosquitofish becomes extinct the melanic population will also stabilize near the frequencies present at the time the temperature stabilizes. (Supported by Old Dominion University Spring 2013 Undergraduate Research Grant.)

### **Biology with Microbiology and Molecular Biology**

GENOME MUTATION MAPPER: NEXT-GEN BIOINFORMATICS APPLICATIONS FOR VIRAL PATHOGENS DETECTION ESPECIALLY HUMAN ADENOVIRUSES. Amirhossein Shamsaddini, Donald Seto, & Ancha Baranova, School of Systems Biology, College of Science, George Mason University. To date, applying high-resolution genomics and bioinformatics approaches has yielded in-depth and better views of the natural variation of Viral Pathogens. Emerging next-generation sequencing technologies have revolutionized the collection of genomic data for applications in bioforensics, biosurveillance, and for use in clinical settings. However, to make the most of these new data, new methodology needs to be developed that can accommodate large volumes of genetic data in a computationally efficient manner. We present Two Different Applications of Computational Framework to analyze Sequences for rapid species identification and Evolution Patterns Analysis. Software for our approaches is available at <http://ssb.gmu.edu/gmm>.

TRACKING BROWN ADIPOSE TISSUE IN VISCERAL FAT TISSUE OF PATIENTS WITH OBESITY. E. Dadkhah<sup>2</sup>, R. Mehta<sup>1,2</sup>, K. Doyle<sup>1,2</sup>, A. Baranova,<sup>1,2</sup> A. Bircerdinc<sup>1,2</sup> & Z. Younossi<sup>1,2,3</sup>, Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA, & <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital. The Prevalence of obesity is going to increase and is estimated to reach 42% of the population by 2030. Several factors are related to obesity. One of the attractive subjects of researches which is even trying to use as a therapy for obesity these days is brown adipose tissue (BAT). The imbalance between BAT and white adipose tissue (WAT) can induce obesity. BAT promotes energy burning that avoids obesity. In this study, the proportion of BAT to WAT DNA was studied in visceral adipose tissue to find out any relation between the amount of BAT and BMI. Ninety eight fresh fat tissues were collected from obese patients at Inova Fairfax Hospital after collecting consent forms. PCR was performed with 15ng of extracted DNA. As BAT differs from WAT by the large amount of mitochondria, the ratio of mitochondrial DNA to genomic DNA considered as the ratio of BAT to WAT in the fat tissue. Two Cyclin B1 primers (CCNB1a & CCNB1C) and two mitochondrial primers (M12 & M13) were selected as genomic and mitochondrial DNA indicators respectively. C(t) values were normalized using CCNBb (IPC) for genomic and M14 for mitochondrial DNA. Technique optimization efforts are ongoing.

IN VIVO FLORESCENT IMAGING OF HOLLOW POLYMERIC NANOCAPSULES LOADED WITH ALEXAFLOUR 750. Venkata Suresh Patthipati<sup>1</sup>, Christopher Osgood<sup>1</sup>, James Swanson<sup>1</sup>, Ramjee Balasubramanian<sup>2</sup>, Kalpana Mahadevan<sup>2</sup>, & Sangbum Han<sup>2</sup>. <sup>1</sup>Department of Biological Sciences, ODU, Norfolk, VA, <sup>2</sup>Department of Chemistry and Biochemistry, ODU, Norfolk, VA. Nanoparticle based imaging strategies hold significant promise in addressing current diagnostic and therapeutic challenges. However, previous nanomaterials failed to clear effectively from the circulation via the renal route. This work demonstrates the use of highly fluorescent, water-soluble, resorcinarene nanocapsules (122 nm) and their effectiveness in biomedical imaging. These hollow polymeric nanocapsules loaded with Alexa Fluor 750 fluoresce brightly in the urinary bladder of mice under the *in vivo* fluorescent imager indicating that their major route of clearance is through the kidney. TEM analysis of the urine recovered from the mice showed the presence of intact nanocapsules. Such florescent nanocapsules can be very effective in visualizing the parts of the urinary tract. They are particularly useful in abdomino-pelvic surgeries ranging from caesarian delivery to laparoscopy. The use of these nanocapsules are not restricted to the imaging as they could be loaded with a host of molecules making them efficient drug delivery vehicles in treating cancer and other pathologies.

THE DIFFERENTIAL ROLE OF CBP UBIQUITIN LIGASE ACTIVITIES IN p53 REGULATION. Oluwatoyin E. Akande & Steven R. Grossman, Dept. of Microbiology & Immunology, Virginia Commonwealth Univ., Richmond, VA 23298. The acetyltransferase CREB-Binding Protein (CBP) is a known transcriptional co-activator involved in p53 regulation and has been shown to encode cytoplasmic, but not nuclear, E3 autoubiquitination and p53-directed E4 ubiquitin ligase activities. In this work, we sought to determine the regulation of differential ubiquitin ligase activities between nuclear and cytoplasmic CBP. Understanding how CBP regulates p53 ubiquitination and stability may lead to potential therapeutics that can modulate p53 stability in cancer cells. We show that a nuclear interacting factor represses cytoplasmic CBP E3 (autoubiquitination) ligase activity in unstressed U2OS cells. We incubated immunoprecipitated cytoplasmic CBP from U2OS cells with either nuclear lysates or CBP- immunodepleted nuclear fraction. Our results showed reduced CBP E3 autoubiquitination in the mixtures when compared to purified cytoplasmic CBP alone. We further showed that DNA damage by doxorubicin (dox) in U2OS cells activated E3 ligase activity in the nuclear fraction. These observations may provide a possible explanation for the inactive nuclear E3 ubiquitin ligase activity but active cytoplasmic E3 and p53-directed E4 ubiquitin ligase activities of CBP in unstressed cells. The dox dependent activation of nuclear CBP E3 autoubiquitination may result from dox dependent inactivation or re-localization of the nuclear inhibitor to other cellular compartments. Further work will identify the nuclear interacting factor and in parallel, determine if the differential ubiquitin ligase activity observed between cytoplasmic and nuclear CBP is otherwise dependent on post-translational modifications of CBP itself.

ENRICHMENT OF CAPTIVE SQUIRREL MONKEYS. LaCheryl A. Ball & Eric L. Walters, Department of Biology, Old Dominion University, Norfolk, VA 23529. Food enrichment is a technique used by the zoo industry to promote overall wellness of animals in captivity. I measured responses of captive, *Saimiri sciureus* squirrel



monkeys to food enrichment at the Virginia Zoo (Norfolk, VA). The research involved determining pre-treatment activity levels in order to test the effect of food enrichment on post-treatment activity levels. I hypothesized that foraging and active behaviors would increase as follows: baseline < post-enrichment < treatment. The experiment was divided into three phases: the first of which provided baseline data on the population's behaviors and activity levels prior to enrichment. The second phase involved the introduction of enrichment feeders on alternating treatment and control days. The third phase involved gathering post-treatment behavioral data, which determined if there were any protracted effects of food enrichment on behavior after the feeders were removed. Ultimately, introduction of food enrichment resulted in a 21% increase in foraging behaviors of both the adult male and juvenile males and a 16% increase in the adults females. In conclusion, food enrichment was a successful method of promoting foraging behaviors and increasing activity levels in captive squirrel monkeys and has important implications for increased health and well-being of captive primates.

EPIGENETICS AND ALZHEIMER'S DISEASE: DISTINCT PATTERN SHIFTS IN DNA METHYLATION. Noor M. Taher, Courtney A. McKenzie, Rebecca C. Garrett, Matthew S. Baker & Gary D. Isaacs, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. Amyloid beta (A $\beta$ ) plaques are one hallmark of Alzheimer's disease. Despite ongoing research, there remains some ambiguity surrounding the role of A $\beta$  in the pathogenesis of this neurodegenerative disease. Even more obscure, however, are the epigenetic changes these plaques cause to neurons. To that end, we wanted to shed more light on the changes in DNA methylation neurons incur when treated with A $\beta$  in vitro. In order to accomplish this, we isolated DNA from A $\beta$ -treated and control neurons and differentially digested the two samples with either a methylation-sensitive or a methylation-insensitive restriction endonuclease. Amplified fragments were then co-hybridized to a commercial promoter microarray. Data analysis revealed a subset of genomic loci that shows a significant change in DNA methylation following A $\beta$  treatment. After mapping these loci to nearby genes, we discovered high enrichment for cell-fate genes that control apoptosis and neuronal differentiation. Finally, we incorporated these genes in a possible model suggesting the means by which A $\beta$  contributes to the brain shrinkage and memory loss seen in Alzheimer's disease.

CHARACTERIZATION OF *CITROBACTER RODENTIUM* TRANSMISSIBLE COLONIC HYPERPLASIA AND IMMUNE RESPONSES IN STREPTOMYCIN-TREATED AND CONVENTIONAL MOUSE MODELS. M. W. Canfarotta<sup>1</sup>, M. H. VanTil<sup>1</sup>, D. A. DeWitt<sup>1</sup>, T. A. Snider<sup>2</sup> & A. J. Fabich<sup>1</sup>, <sup>1</sup>Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502 & <sup>2</sup>Center for Veterinary Health Sciences, Oklahoma State University. *Citrobacter rodentium* is an enteric murine pathogen similar to the human pathogen enterohemorrhagic *Escherichia coli* (EHEC) as it forms an attaching and effacing (A/E) lesion during gastrointestinal infection. Previous studies have utilized *C. rodentium* as a mouse model for EHEC disease in humans, however there is currently no comprehensive study characterizing the course of infection in specific mouse models with or without streptomycin treatment. The use of streptomycin effectively depletes the facultative anaerobic niche allowing for the direct study of *C. rodentium* colonization, whereas a model lacking antibiotic treatment

studies pathogenesis since *C. rodentium* must overcome colonization resistance. In this study, *in vivo* colonization assays of *C. rodentium* were performed in CD-1, BALB/c, C57Bl/6, and C3H/Hen mice with and without streptomycin treatment and colon health was monitored histologically and quantitatively by measuring empty colon weight to identify edema and hyperplasia. Male CD-1 mice with streptomycin treatment were colonized by *C. rodentium* at stable levels without severe colonic inflammation, whereas male C3H/Hen mice without antibiotic treatment displayed the most significant colonic hyperplasia, suggesting that these models are best utilized to study colonization and pathogenesis respectively. This study was funded in part by The Virginia Academy of Science.

**SYNERGISTIC AND ANTAGONISTIC EFFECTS OF PESTICIDE MIXTURES TO BEES.** J. R. Williams, R. D. Fell & T. D. Anderson, Dept. of Entomology, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Pollinators are a critical component to plant health and production of agricultural landscapes. Pesticide exposures to honey bees, *Apis mellifera* L., are implicated in the decline of these pollinators and their ecosystem services. Knowledge of the toxicological consequences of these pesticide exposures, alone and in combination, to bees and their decline is limited. In the present study, we will show the: 1) acute toxicities of in-hive pesticides to bees; 2) binary interactions of in-hive pesticides to bees; and 3) metabolic activities of bees towards in-hive pesticides. Select pesticides (*tau*-fluvalinate, coumaphos, coumaphos-oxon) are acutely toxic to bees at environmentally relevant levels of parts per billion (ppb), an order of magnitude lower than those concentrations reported in bee colonies. The combination of *tau*-fluvalinate and antibiotics increases bee mortality *ca.* 50%; however, a combination coumaphos-oxon and antibiotics reduces bee mortality *ca.* 20% relative to the miticide-only treatments. In contrast, the combination of *tau*-fluvalinate and chlorothalonil reduces bee mortality *ca.* 27%; whereas, a combination of coumaphos-oxon and chlorothalonil increases bee mortality *ca.* 30% relative to the miticide-only treatments. The exposure of bees to antibiotics or chlorothalonil reduces cytochrome P450 monooxygenase activity *ca.* 50%; whereas, esterase and glutathione *S*-transferase activities increase *ca.* 26% relative to the untreated bees. This information will be utilized for the development of ecologically- and chemically-based strategies to improve pollinator health and reduce pesticides exposures to bee colonies in southwest Virginia.

**EVIDENCE OF P-GLYCOPROTEIN MODIFICATION OF INSECTICIDE TOXICITY IN VECTOR MOSQUITOES.** Ngoc N. Pham & Troy D. Anderson, Department of Entomology, Virginia Tech, Blacksburg, VA 24061. Mosquitoes affect human health worldwide as a result of their ability to vector multiple diseases. Widespread resistance is a serious public health challenge that limits the use of high efficacy insecticides to reduce the risk of mosquito-vectored diseases. P-glycoprotein (P-gp) is an efflux transporter that assists in maintaining the blood-brain barrier interface of insects and may serve as a first line of defense to insecticide exposures. Previous studies have demonstrated the blood brain-barrier of mosquitoes to interfere with target-site action of established and experimental insecticides; however, the interaction of P-gp toward these chemistries is unclear. In this study, we provide a: 1) toxicological analysis of tacrine-based anticholinesterases for mosquitoes, alone and

in combination, with the P-gp inhibitor verapamil and 2) biochemical analysis of acetylcholinesterase for mosquitoes exposed to these compounds.

E3-MEDIATED DOWN-REGULATION OF THE CIRCADIAN FACTOR PERIOD 2. Jing-Jing Liu, Tetsuya Gotoh, Marian Vila-Caballer, Carlo S. Santos, Jianhua Yang, & Carla V. Finkielstein, Department of Biological Sciences, Virginia Tech, Blacksburg, VA. The circadian rhythm and cell cycle are the two main oscillatory systems in cells. How cells sense time and decide what is the best time for growing, proliferating or apoptosis? One possibility is that there are crosstalks between these two systems. Based on the fact that Period 2 (Per2) also plays essential role in DNA damage response, Per2 is supposed to connect circadian rhythm and cell cycle, which makes Per2 work as a tumor suppressor. We found Per2 regulating p53 pathway but little is known about how Per2 itself is regulated. One interesting finding is that independent of transcriptional regulation, overexpressed Per2 protein also oscillates; this implies posttranslational modifications are essential for sustaining Per2 protein oscillation. Per2 binds to Mdm2, a well-known E3 ubiquitin ligase, both *in vitro* and *in vivo*. Mdm2 induces Per2 ubiquitination *in vitro*, but further experiments are needed to verify Mdm2 is an E3 ligase for Per2 *in vivo*.

CIRCADIAN MODULATION OF CELL CYCLE PROGRESSION. Samuel Schiffhauer, Marian Vila-Caballer, Tetsuya Gotoh, Jianhua Yang & Carla V. Finkielstein, Department of Biology, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Individuals who have prolonged disrupted circadian rhythms, such as shift workers and flight attendants, display higher rates of cancer development. Yet the impact of the body's circadian rhythm on critical cellular processes such as cell division is complex and poorly understood. We aim to develop an overall representation of the influence circadian proteins have on normal cell cycle progression. To achieve this, cytosolic levels of cell cycle proteins were monitored over a 48 hour period in a human cancer cell line (HCT116), then compared to an identical experiment in which circadian regulator protein Period 2 was overexpressed. Shifts in both amplitude and timing were observed in Cyclin E, Cyclin B, phospho-Cdc2, Wee1, and p21, supporting our hypothesis that circadian proteins are capable of direct control over cell cycle progression in unstressed cells. Furthermore, we showed critical circadian oscillator proteins CLOCK, BMAL1, Per2 and Cry1 have direct transcriptional activation activity on the promoter regulatory elements of the kinase inhibitor p21, a protein with well established roles in cellular senescence, polyploidy, and differentiation. The consequences of this activation in a cancer cell model will be the focus of future experiments, to better understand the molecular underpinnings of cancerous phenotypes.

PRIMER DESIGN AND VALIDATION FOR REFERENCE GENES AND ALTERNATIVELY SPLICED MRNAS ENCODING FOR RAT CGRP AND CALCITONIN. Caitlin Koob<sup>1,2,3</sup>, Katie Doyle<sup>1,2</sup>, Rohini Mehta<sup>1,2</sup>, Aybike Biredinc<sup>1,2</sup>, Margaret Slavin<sup>4</sup> & Ancha Baranova<sup>1,2</sup>, <sup>1</sup>Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, College of Science, George Mason University, Fairfax, VA. <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, <sup>3</sup>Chemistry Department, College of Science, George Mason

University, Fairfax, VA, & <sup>4</sup>Department of Nutrition and Food Studies, College of Health and Human Services George Mason University, Fairfax, VA. Peripheral CGRP release is a marker for neurogenic inflammation and a contributor to migraine headache. Orthologous *Cgrp* and calcitonin encoding mRNAs expressed in rat thyroid parafollicular CA77 cells may provide a model for the screening of migraine-provoking food compounds. Designing primers for alternatively spliced *Cgrp* variants and normalizing a set of reference genes specific to this cell line is necessary to measure expression of *Cgrp* by qPCR. In this study, all reference mRNAs were successfully validated by qPCR using Rat Universal and rat thyroid parafollicular CA77 cDNA as templates and yielded predicted product size. The *Cgrp* primers did not show amplified product in Rat Universal, this may be because it does not contain the mRNA sequence for *Cgrp* that is known for its expression in only certain types of cells. *Cgrp* variants did show amplification in rat thyroid parafollicular CA77 cDNA, but optimization of primer annealing temperature and concentration is necessary. Future work will include normalizing validated reference mRNAs and selecting two genes that will serve as internal controls when measuring *Cgrp* expression in migraine models by qPCR.

THE KCTD FAMILY OF GENES AS POSSIBLE REGULATORS OF THE ADIPOSE TISSUE FUNCTION IN MORBID OBESITY. Mariam Hashemi<sup>1,2,3</sup>, Ancha Baranova<sup>1,2</sup> & Aybike Biredinc<sup>1,2,1</sup> Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & <sup>3</sup>Biology Department, College of Science, George Mason University, Fairfax, VA. KCTD is a family of tetramerization (T1) domain proteins that are similar to T1 domains of the voltage-gated channels. It has been predicted that KCTD may interfere with the assembly of the K<sup>+</sup> channels by binding to their own T1 domain. A previous study has demonstrated KCNRG, a KCTD protein member, causes voltage-gated suppression. Visceral adipose tissue (VAT) samples were used in this study due to its endocrine-signaling characteristics. The purpose of this study is to determine if there are any genes in the KCTD protein family that are involved in diet-induced obesity. A literature research was conducted using PubMed to obtain any data on association between KCTD proteins and obesity or liver function. Primers were designed for the following KCTD proteins: KCTD9, KCTD12, and KCTD15. Using the VAT samples RNA extraction was conducted and converted to cDNA and stored for further use of qPCR and gene expression.

A STUDY OF FROZEN WHOLE TISSUE PRESERVATION FOR CELL SURVIVABILITY. Scarlett Koga<sup>1,2,3</sup>, Ancha Baranova<sup>1,2</sup> & Aybike Biredinc<sup>1,2</sup>, <sup>1</sup>Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & <sup>3</sup>Biology Department, College of Science, George Mason University, Fairfax, VA. Visceral fat (visceral obesity) is a main contributor to the development of metabolic syndrome. Metabolic syndrome is a variety of medical disorders that may increase an individual's chance of developing cardiovascular disease alongside with other health complications such as diabetes or stroke. One of the major limitations in the study of human adipose sample is a variation of the tissue preservation techniques which may cause various degrees of RNA degradation, thus

limiting the value of downstream gene expression studies. For the purpose of this study, a literature survey of various methods for whole tissue preservation was conducted to find techniques to maximize the potential shelf-life of human adipose tissue to validate preservation techniques, we will use pre-existing adipose tissue samples that differ in the time of their storage at -80°C. An online survey with PubMed and Google was completed to find methods of preservation offered commercially. Hypothermic and Cryogenic preservations are common methods to preserve human adipose tissue. These preservation methods will be used to store samples and PCRs will be run to determine the degree of RNA degradation between the two techniques.

#### Posters

EFFECTS OF BRAIN DERIVED NEUROTROPHIC FACTOR ON Y79 RETINOBLASTOMA CELLS. Andrew N. Hogan & Rosemary Barra, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22405. Retinoblastoma is a rare form of cancer that affects the retina of the eye, occurring in approximately 1 in 15,000 live births, and it is also the most common type of inherited malignancy. Although current treatments including chemotherapy and surgical procedures have been successful, there is still a high rate of reoccurrence in patients. Recent research on neural cancers has focused on the neurotrophin receptors and their corresponding ligands. This study was designed to evaluate the role of the p75 neurotrophin receptor (p75NTR) in retinoblastoma cells. The receptor and its ligand are known to play a role in the regulation of programmed cell death in neural tissues. Brain derived neurotrophin factor (BDNF) is a neural ligand typically associated with neurotrophin receptors. Retinoblastomas have been found to express low levels of p75NTR, leading to an increase in cell growth. The primary cell line used in this study was the Y79 human retinoblastoma cell line. The cells were cultured in RPMI media with 10% fetal bovine serum, and 1% penicillin-streptomycin. Initial immunocytochemical studies using an anti-p75 NGF receptor antibody (ab8874) demonstrated the presence of the p75NTR on the retinoblastoma cells. The Y79 cells were incubated for 24 hours at 37°C with various concentrations of BDNF (0.02ng/mL, 0.2ng/mL, and 2.0ng/mL) and a MTT viability assay was performed. Contrary to current literature, the MTT assay showed a decrease in overall cell viability at all concentrations tested. Treatment with 2.0 ng/ml BDNF decreased cell viability by 60% compared to the control cultures. A human 3 – caspase ELISA assay was conducted to determine whether the decrease in viability was caused by caspase 3 mediated apoptosis. Preliminary results indicate that BDNF treatment does affect caspase 3 activity. Cells incubated with 4 ng/ml of BDNF for 24 hrs showed a 30% decrease in caspase-3 activity.

INSIGHTS INTO THE COMPLEX FORAGING ECOLOGY OF THE APPALACHIAN COYOTE (*CANIS LATRANS*) USING STABLE ISOTOPE ANALYSES. P. A. P. deHart, C. B. Shutt, & R. Scruggs, Department of Biology, Virginia Military Institute, Lexington, VA 24450. The coyote (*Canis latrans*) has increased in population throughout the east coast of North America, especially in the recent decade. As a consequence of this range expansion, human-coyote interactions have increased, directly through livestock predation and indirectly through resource

competition with local hunters (primarily deer). Effective management of these interactions has been hampered by a significant lack of knowledge of the basic foraging ecology of coyotes. To investigate the specific trophic role of the coyote in this ecosystem, we examined the carbon and nitrogen isotopic signature from the hairs of wild coyotes and potential prey throughout Appalachia in multiple habitats. We found that coyotes varied widely in both  $\delta^{15}\text{N}$  (5.9-10.6‰) and  $\delta^{13}\text{C}$  (-25.5-21.1‰), highlighting foraging on multiple trophic levels and likely dependent upon regional differences in prey availability. Additionally, while concurrent studies suggest a seasonally shifting dependence on deer, mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from coyotes captured over all seasons are consistent with a diet more predictably dependent on communities of small mammals. This new evidence suggests that while some coyotes may be occasionally utilizing select livestock as prey, they display greater foraging dependence on available wild species found in forested habitats. This study yields some of the first evidence into the true diet of coyotes in this region and also provides essential baseline values for multiple mammals in Virginia. This research suggests that regional variations in coyote predation strategy be considered to both refine current management plans and more efficiently implement the conservation plans for endangered competitors and prey.

**NKG2D RECEPTOR ACTIVATION OF MTOR AND NFkB ENHANCES PROINFLAMMATORY CYTOKINE SECRETION IN CD8<sup>+</sup> T CELLS.** E. A. Whitman, T. Bedsworth & A. Barber, Department of Biological and Environmental Sciences, Longwood University, Farmville, VA, 23909. To induce strong anti-tumor immune responses, CD8<sup>+</sup> T cells require stimulation through the TCR and costimulatory receptors. Each costimulatory molecule can have a unique effect on the function of T cells due to differential activation of downstream signaling pathways and gene expression. One costimulation molecule that is likely to be engaged in the tumor microenvironment is NKG2D since the ligands for this receptor are expressed in over 80% of tumors. In order to determine how activation of costimulatory receptors in the tumor microenvironment affects CD8<sup>+</sup> T cell functions, this study investigated the differential activation of signaling pathways by two costimulatory receptors, CD28 and NKG2D. Specifically, this study focused on the activation of mTORC1 and mTORC2 along with NFkB, all of which have been shown to have important roles in anti-tumor functions of CD8<sup>+</sup> T cells. Activation of CD8<sup>+</sup> T cells through CD3 and NKG2D lead to an increased secretion of proinflammatory cytokines and a decrease in anti-inflammatory cytokines compared to CD3 or CD3/CD28 stimulation. These data show that stimulation through NKG2D leads to the differential activation of signaling pathways and alters the anti-tumor effector functions of CD8<sup>+</sup> T cells.

**IMPROVING EFFICIENCY OF GENOMIC SEQUENCING FROM DIVERSE NEMATODE SPECIES.** Anna K. Kania, Daniel E. Browne, & Theresa M. Grana, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Even though nematodes are one of the most abundant phyla on the planet, their phylogenetic history is lacking a lot of information due to the fact that only few thousand out of the estimated million species have been identified and characterized. Thus to truly understand the evolutionary patterns that have taken place in the very large and diverse phylum of Nematoda, more species need to be described and

sequenced. The primary goal of this project is to improve the efficiency and quality of genomic sequencing of new nematode species. The DNA sequences obtained through the process are used to place those new species in context of their evolutionary history on the phylogenetic tree. The genomic sequencing process is challenging, however. It consists of multiple steps, each with alternative ways of accomplishing the same task. The principal objective is to determine the optimal way of accomplishing each and every step and therefore maximize the overall efficiency. While progress has been made and now we are able to obtain sequences from 60% of strains, further improvements are necessary. Our goal is to obtain a good DNA sequence from 90% of the new nematode species collected.

**A STUDY OF CHLOROQUINE'S ANTIRETROVIRAL CHARACTERISTICS.** Ryan S. Green & Lynn O. Lewis, Dept. of Biological Sciences, Univ. of Mary Washington, Fredericksburg, VA, 22401. In this study a procedure to induce maximal viral replication was tested, in addition to testing chloroquine as a possible treatment. This research attempted to activate Murine Mammary Tumor Virus (MMTV) using progesterone, as opposed to the standard synthetic activator that is used, to increase viral yield (Ross, 2010). Additionally viral infections are difficult to treat. This research examined the effects of chloroquine, an anti-malarial drug, as a retroviral treatment. Chloroquine has been shown to have antiviral properties; however, its effects have not been adequately studied in retroviruses and never with MMTV (Campitelli, et al., 2007). It was hypothesized that if chloroquine were to be administered to MMTV infected tissue culture, then the cytopathic effects (CPE) due to MMTV would be reduced. During this study it was found that progesterone, corticosterone, and dexamethasone did increase CPE (visible morphological changes which can indicate viral replication and transformation) at high concentrations, but the different treatments had no significant difference. The lack of difference was possibly due to the range between concentrations being too large to pinpoint the differences between the similar chemicals. Chloroquine had no significant effect on dexamethasone activated cells, possibly due to too few trials to demonstrate the expected trends, or viral budding masking CPE due to viral integration. This research was funded by the University of Mary Washington and the Virginia Academy of Science.

**OBJECT NEOPHILIA IN PUREBRED DOMESTIC DOGS.** Lydia B. Kniewski & Gene Sattler, Dept. of Biology & Chemistry, Liberty Univ., Lynchburg, VA 24502. Neophilia is defined as a preference for novelty, and has been studied in a variety of animal species. We tested two purebred dog breeds for neophilia with inanimate objects. Observations of dogs' selection when presented with two familiar toys and a novel toy were analyzed. Novel toys were preferred in 60% of selections presenting a significant neophilic trend ( $p=0.002$ ). Of the breeds analyzed, Labradors selected novel toys 53% of the time, while Brittany's preferred them in 67% of selections. Although both breeds showed a neophilic trend, only in Brittany's was it significant ( $p=0.009$ ). Differing degrees of neophilic tendency may exist among breeds as a result of either past or current selective regimes. These tendencies may have played a role in the domestication of dogs, and could lend insight into breed characteristics.

PROGRESS ON THE RAI1 BINDING SITE IN *CLOCK*. K. Belrose-Ramey<sup>1</sup>, C. Bax<sup>1</sup>, S. Williams<sup>2</sup>, S. Mullegama<sup>2</sup>, S. Elsea<sup>2,3</sup>, & D. Zies<sup>1</sup>, <sup>1</sup>Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401, <sup>2</sup>Virginia Commonwealth University, Richmond VA 23284, <sup>3</sup>Baylor College of Medicine, Houston, TX 77030. Smith-Magenis Syndrome (SMS) is a neurobehavioral disorder characterized by mental retardation, sleep disturbances, obesity and attention seeking behavior. The major symptoms of SMS are caused by haploinsufficiency of the *retinoic acid-induced 1 (RAI1)* gene, a transcription factor involved in the regulation of many genes. Previously, a Chromatin Immunoprecipitation-microarray chip (ChIP-chip) experiment was conducted to determine genes regulated by RAI1. One gene identified was *Circadian Locomotor Output Cycles Kaput (CLOCK)*. The region of *CLOCK* present in the microarray chip was used to create a pGL3 luciferase reporter gene construct. Previous studies showed that transcription from this construct was increased when cotransfected with RAI1, suggesting RAI1 binding site was located within the fragment. Site directed mutation, serial deletions, and luciferase analysis led to the identification of a 50bp region containing the RAI1 binding site. Possible false positives with the pGL3 reporter gene led to the decision to move an updated reporter system creating pGL4-*CLOCK*(-). The goals of my project have been to re-create constructs in the pGL4 system and compare the results between the two systems. Once completed, I will continue with serial deletions and perform single-base mutations to identify the minimal binding sequence for RAI1. The identification of the RAI1 binding site would enable researchers to identify other genes regulated by RAI1 and could lead to new treatments for SMS.

THE EFFECTS OF BISPHENOL A ON Wnt GENE EXPRESSION IN DEVELOPING RATS. Kara Arbogast, Virginia L. King & Deborah A. O'Dell, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Wnt proteins are involved in development of the nervous and reproductive systems in vertebrates and aberrant expression of certain Wnt proteins has been linked to cancers of the reproductive systems in males and females. The Wnt pathway has been demonstrated to interact with the estrogen pathway, indicating that stimulation of the estrogen pathways may affect the transcription of Wnt proteins. Bisphenol A (BPA) is a xenoestrogen which interacts with estrogen receptors, and has been linked to abnormal development and cancers of the reproductive systems. We examined whether the Wnt pathways could be affected by exposure to BPA during development. Tissues from neonates from mothers fed BPA(100 mcg/L) during gestation were compared with tissues from neonates of untreated mothers. Brains, gonads and reproductive tracts were analyzed for levels of Wnt7a and Wnt 5a, using Sandwich ELISA and for levels of BPA using HPLC. BPA levels in embryonic tissues in general were higher in treated than in untreated tissues. ANOVA analysis did not show any significant differences in Wnt proteins between treated and control tissues. Post-hoc analysis using Fishers LSD test did show significant differences in Wnt 7a levels in the male reproductive tract and brain and in Wnt 5a levels in the male reproductive tract. BPA can therefore interfere with normal reproductive tract formation by altering Wnt gene activity.



EPIGENETIC REMODELING AT DISCRETE GENOMIC LOCI IN A $\beta$ -TREATED NEURONS. Matthew Baker, Noor Taher, Courtney McKenzie, Rebecca Garret & Gary D. Isaacs, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA. Genetic contributions leading to the development of Alzheimer's disease are mostly obscure, however evidence suggests that epigenetic modifications may play a role. Cytosine methylation has been shown to play a role in several biological disease states, and several AD-associated genes are regulated through this mechanism. Therefore, we set out to determine if methylation can provide genetic markers for AD development. DNA was isolated from A $\beta$ -induced neuronal cells from an IMR-32 cell line, which serves as the AD model, as well as non-induced control neuronal cells. Using methylation sensitive (HpaII) and insensitive (MspI) restriction endonucleases, which both target the sequence 5'-CCGG-3', we determined the methylation status of genomic promoter regions through LM-PCR and subsequent microarray analysis. Genomic regions showing A $\beta$ -induced methylation modifications were then narrowed down to the 0.1% most changing regions for stringency purposes. The regions showing significant change were linked to their ontological function, revealing significant correlation with ontologies related to neurogenesis and apoptosis. These findings support the idea that AD pathology is associated with specific epigenetic changes, and that these changes influence specific cellular pathways. This research was funded by the Jeffress Memorial Trust [J-998].

ANALYSIS OF 5-HYDROXYMETHYLCYTOSINE DISTRIBUTION IN THE HIPPOCAMPUS OF AN ALZHEIMER'S MOUSE MODEL. Rebecca Haraf, Deanna Harrison, Matthew Baker, Noor Taher, & Gary D. Isaacs, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA. Methylated and hydroxymethylated cytosines are epigenetic modifications known to directly affect gene expression. Although methylated cytosines have previously been associated with Alzheimer's Disease (AD), they have been primarily studied on a gene by gene basis. The genome-wide roles of the modifications of cytosine, specifically hydroxymethylation, are still unclear. DNA from the hippocampus of an AD mouse model was purified and a DNA Immunoprecipitation (DIP) assay done using antibodies specific to pull down to hydroxymethylated cytosines (hmCs). The DNA was hybridized to a microarray with 20,404 promotor regions and 15,980 CpG islands. This gave significant peaks of hmC gain or loss in the experimental mouse when compared to the control model. We used the data to determine regions that had a change in hmC content when an AD state was established. Our confirmation DIP will show that AD is an epigenetic disease specifically affected by the distribution and levels of hmC in the hippocampus of a mouse model. We will analyze these changes to determine the specific cell functions and biological pathways affected by hmCs in Alzheimer's Disease. Funded by Jeffress Memorial Trust [J-998].

BACTERIOPHAGE-MEDIATED ANTIBIOTIC RESISTANCE. Kathleen O. Blevins & Lynn O. Lewis, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Bacteriophages, viruses that infect a specific host range of bacteria, can have one of two different replication cycles. The lysogenic cycle incorporates the phage genome into the host cell's genome after infection, while the lytic cycle produces more viruses and kills the host cell by lysis. As phages are

particular to only one type of bacteria, they could be used as an alternative method to treat antibiotic resistant bacterial infections, which is known as phage therapy. However, during the lysogenic process, phage genes could be transferred from phage to bacteria, making phage therapy risky. Phages that were isolated on *Bacillus thuringiensis* Kurstaki were sequenced, and a beta-lactamase gene was identified. These phages were used to lysogenize the nonpathogenic *Bacillus anthracis* Delta Sterne and tested for the transfer of antibiotic resistance by the Kirby Bauer test. No antibiotic resistance was noted in the lysogens. Because we have now isolated a number of *Bacillus* phages, a simple method for classifying them was attempted by utilizing PCR and primers designed to match several specific phages. The 37 phages isolated by the 2012-13 Phage Hunters class were separated into 15 subcategories based on the primer reactions, and the phages which had been sequenced were verified with BLAST. Further, one of the sequenced genomes, belonging to *Bacillus thuringiensis* Kurstaki phage JPB9, was annotated.

IN VITRO SELECTION OF *E. COLI* MG1655 *flhDC* MUTANTS. Ryan N. Montalvo, Edward Dunn Richards & Andrew J. Fabich, Department of Biology and Chemistry, Liberty University. Leatham *et al.* previously reported that *Escherichia coli* strain MG1655 loses motility after three days in the streptomycin-treated mouse intestine. MG1655 has been previously evaluated for motility but HS and Nissle human commensal strains have not. Motility research carried out by Fabich, Leatham, Gauger *et al.* has been known to last for a period of 15 days, while our experiment is ongoing past 60 days. In order to evaluate motility the mechanism by which motility is lost needed to be identified. When sequenced, these intestine adapted strains (\*) displayed deletions of various lengths downstream of the *IS1* element within the regulatory region of the *flhDC* operon, which encode the master regulator for flagellar synthesis. In our study of this mutation we observed trends within the data where the strains exhibit gains or losses in motility on similar days, specifically with MG1655 and HS. MG1655 is an average of 33.7% less motile than the other strains consistently. On day 60, the three strains were less than 60% motile and ranged within 36.11% of one another. This data at 60 days would suggest that the strains may reach an relative equilibrium when examined for further months. The similar trends in fluctuation of motility amongst the individual strains supports the idea that motility within the intestine is a dynamic contributing factor to *E. coli* intestinal colonization niches.

THE GUT REACTION: HOW THE INTESTINAL MICROBIOTA RESPOND TO COLONIZATION OF *CITROBACTER RODENTIUM*. C. E. Black & A.J. Fabich, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. Pathogenic *Escherichia coli* O157:H7 is a global health threat. The microbial response to enteric colonization of *E. coli* O157:H7 is not well understood, nor the potential role the intestinal microbiota in preventing its disease. By using the streptomycin-treated mouse model, streptomycin-resistant bacteria *Citrobacter rodentium*, as well as commensal *E. coli* strains MG1655 Str<sup>r</sup> Nal<sup>r</sup> and probiotic Nissle1917 Str<sup>r</sup> Nal<sup>r</sup>, were colonized for community analysis. We hypothesize that *Bacteroidales* spp. and *Clostridium* spp. will have significantly altered population abundances when streptomycin-treated mice are co-colonized with *C. rodentium* and either commensal *E. coli* strain MG1655 Str<sup>r</sup> Nal<sup>r</sup> or the probiotic Nissle 1917 Str<sup>r</sup> Nal<sup>r</sup>. Quantitative PCR

was used to determine the bacterial abundance and the complex interactions occurring with inoculation of *C. rodentium* and either MG1655 or Nissle. The results indicate an increase in the population abundance of *Bacteroidales* spp. with the co-colonization of Nissle but not MG1655; an increase in the population abundance of *Clostridium* spp. with the co-colonization of MG1655 and Nissle; and a 5-fold increase of *Lactobacilli* spp. with the co-colonization of MG1655. These results shed light on the competitive relationship between pathogenic *E. coli* O157:H7 and specific enteric microbes as well as the protective roles MG1655 and Nissle may have against the pathogen.

CHICKENS ARTIFICIALLY SELECTED FOR HIGH (HWS) AND LOW (LWS) BODY WEIGHT DIFFER IN PANCREATIC EXPRESSION OF GLUCOSE REGULATORY GENES. L. H. Sumners<sup>1</sup>, X. Zhao<sup>2</sup>, W. Zhang<sup>1</sup>, C. F. Honaker<sup>1</sup>, P. B. Siegel<sup>1</sup> & E. R. Gilbert<sup>1</sup>, <sup>1</sup>Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061 and <sup>2</sup>Sichuan Agriculture University, Ya'an, China. Now in the 55<sup>th</sup> generation of selection, chickens genetically selected for high (HWS) and low (LWS) juvenile (8 wk) body weight differ in weight by 12-fold at selection age. Because HWS chickens display impaired glucose tolerance and a difference in threshold response to insulin, compared to LWS, we hypothesized that the lines differ in mRNA abundance of glucose regulatory genes. Pancreas head and body were sampled from male and female HWS and LWS chickens (day 65: n = 10/line), and mRNA of preproinsulin (*PPI*), preproglucagon (*PPG*), glucose transporter 2 (*GLUT2*), and pancreatic and duodenal homobox 1 (*Pdx1*) were measured by real-time PCR (2<sup>- $\Delta\Delta C_t$</sup>  method). Effects of line, tissue, sex and the interactions between them were analyzed by ANOVA, and means were separated by Tukey's HSD. For all genes, mRNA was greater (p < 0.01) in LWS. *PPI* mRNA was greatest in the pancreas body of LWS males (p = 0.009), whereas *PPG* mRNA levels were lowest in the pancreas body of all male chickens (p = 0.01). Abundance of *GLUT2* mRNA was greater (p = 0.001) in males than females. *Pdx1* mRNA was more abundant in the pancreas body of females than of males, and the pancreas head of females (p = 0.002). There was also greater *Pdx1* mRNA in both the pancreas head and body of LWS, than HWS chickens (p = 0.04).

DEFINING DIRECT TARGETS OF OpaR IN THE QUORUM-SENSING REGULON OF *VIBRIO PARAHAEEMOLYTICUS*. A. L. Kernell<sup>1</sup>, L. T. C. Guthrie<sup>1</sup>, R. V. Jensen<sup>1</sup>, L. L. McCarter<sup>2</sup>, & A. M. Stevens,<sup>1,†</sup> Department of Biological Sciences (MC0910), 1070 Washington St. SW /Rm 222 Life Sciences I, Virginia Tech, Blacksburg, Virginia 24061; <sup>2</sup>University of Iowa, 3-430 BSB Iowa City, IA 52242. *Vibrio parahaemolyticus* (VP) is an emerging pathogen that is associated with food-borne illnesses worldwide, including developed countries, such as the United States and Japan. It is acquired through consuming raw or undercooked seafood. The pathogenesis of this organism is controlled by the phenomenon known as quorum sensing and the master regulator of quorum sensing, in (VP), is OpaR. OpaR controls the virulence of (VP), as well as the colony and cellular morphology associated with growth on a surface. RNA-Seq, whole transcriptome Next Generation sequencing, was utilized to determine all the direct and indirect targets controlled by OpaR and revealed that over 10% of the genome is regulated by quorum sensing. Eleven transcription factors under OpaR control were identified and further studied using qRT-PCR and the

data collected confirmed the RNA-Seq results. ChIP-Seq methods are being optimized to identify the direct targets of OpaR. Select targets were confirmed direct targets via *in vitro* electrophoretic mobility shift assays (EMSA) with purified hexahistidine tagged OpaR. A previously published position specific weighted matrix (PSWM) will be utilized to assist in identification of possible OpaR binding sites.

**DIVERGENCE OF X CHROMOSOME HETEROCHROMATIN AMONGST SPECIES OF THE ANOPHELES GAMBIAE COMPLEX.** Atashi Sharma, Maria Sharakhova & Igor V. Sharakhov, Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA - 24061. Malaria is a scourge infecting 215 million people annually. The vector mosquito *Anopheles gambiae* along with its six sibling species forms the *gambiae* complex. Major vector *Anopheles gambiae* sensu stricto (s.s.) is currently undergoing incipient speciation into two molecular forms, designated M and S. Differences between these forms were determined on the basis of heterochromatin (repetitive DNA) present in X chromosomes using light microscopy and Fluorescence *in situ* hybridisation (FISH). In addition, 18S rDNA (responsible for protein synthesis) probe and species specific satellite DNA (important part of heterochromatin) was labelled and mapped to *An. gambiae*. We found significant differences in both the patterns and intensity of heterochromatin between the M and S forms. The 18S rDNA probe mapped adjacent to the heterochromatin in all the laboratory strains tested. Overall, our study indicates differences in heterochromatin between and within the species of *gambiae* complex and suggests a possible role of heterochromatin in speciation and protein synthesis.

### Biomedical and General Engineering

**ELECTROSPINNING AND CHARACTERIZATION OF LINEAR-DENDRITIC COPOLYMERS AS A NEW TISSUE ENGINEERING SCAFFOLD.** Donald Jr C Aduba<sup>1</sup>, Jefferson W. Overlin<sup>1</sup>, Gary L. Bowlin<sup>1</sup> & Hu Yang<sup>1,2</sup>, <sup>1</sup>Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284 & <sup>2</sup>Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298. Polyamidoamine dendrimers (PAMAM) are an important class of macromolecules, which are known for their highly branched nanoscale structures and high density of surface groups. Numerous studies have been conducted to develop PAMAM dendrimer-based nanocarriers to deliver a broad spectrum of functional moieties such as drugs, imaging agents, and ligands. The purpose of this work is to explore a new way of using PAMAM dendrimers for wound healing and drug delivery applications. Particularly, we have developed novel electrospun nanofiber scaffolds made of linear-dendritic copolymer comprised of methoxy-polyethylene glycol (mPEG) as modifier to couple (PEGylate) dendrimer in modulating their biological and surface properties while facilitating electrospun nanofiber formation. In our study, we adjusted the degree of PEGylation of the dendrimer surface groups (100%, 50%, and 12.5%) by attaching PEG at 32:1, 16:1 and 4:1 ideal molar ratios respectively. Crosslinking reagents Eosin Y and 2,2-Dimethoxy-2-phenyl-acetophenone (DMPA) were used to help stabilize the electrospun scaffold. For each treatment, the scaffold's physical properties were evaluated. Ongoing studies include cell viability assays evaluating cell-scaffold

interactions and their response. This research was supported, in part, by NIH R21NS063200 and NSF CAREER Award CBET0954957. D.A. is a recipient of SREB-State Doctoral Fellowship.

**SYNTHESIS AND CHARACTERIZATION OF ANTIEPILEPTIC NANOMEDICINE FOR TRANSBUCCAL DELIVERY.** Donald Jr C Aduba<sup>1</sup>, Olga Y. Zolotarskaya<sup>1</sup>, Gary L. Bowlin<sup>1</sup> & Hu Yang<sup>1,2</sup>, <sup>1</sup>Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284, & <sup>2</sup>Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298. Epilepsy therapeutic treatment is aided with a variety of formulations such as sustained release tablets, sprinkle tablets, delayed release capsules and syrup solutions, which primarily rely on oral administration. Using nanoparticles to deliver central nervous system (CNS) drugs can help enhance brain uptake. However, intravenous injection is often applied for nanoparticle-based therapeutics and likely cause poor patient compliance among patients suffering from such chronic disease as epilepsy. The purpose of this work is to develop antiepileptic nanomedicine and nanofiber formulation and to explore the buccal mucosa as a non-invasive adsorption site for delivery of nanomedicine. Antiepileptic drug valproic acid (VPA) was used as a model drug with highly branched nanoscale polyamidoamine (PAMAM) dendrimer as the underlying carrier to construct nanomedicine. The synthesized VPA nanomedicine, confirmed by <sup>1</sup>H NMR was then loaded to electrospun nanofiber scaffold to make a mucoadhesive formulation designed for transport across the buccal mucosa. This research was supported, in part, by NIH R21NS063200 and NSF CAREER Award CBET0954957. D.A. is a recipient of SREB-State Doctoral Fellowship.

**A CELLULARIZED ELECTROSPUN FIBER/HYDROGEL COMPOSITE MESH FOR LIGAMENT TISSUE ENGINEERING.** P. S. Thayer<sup>1</sup>, A. Dimling<sup>2</sup>, M. R. Hahn<sup>3</sup>, S. A. Guelcher<sup>4</sup> & A. S. Goldstein<sup>1,2</sup>, <sup>1</sup>School of Biomedical Engineering & Sciences, Virginia Tech, Blacksburg VA, <sup>2</sup>Dept. of Chemical Engineering, Virginia Tech, Blacksburg VA, <sup>3</sup>Dept. of Biomedical Engineering, RPI, Troy NY, & <sup>4</sup>Dept. of Chemical & Biomolecular Engineering, VU, Nashville TN. Electrospinning is applied extensively for fabrication of fibrous meshes for research in the development of engineered tissues. While promising due to their fibrous architectures, electrospun meshes suffer from limited overall thickness and cellular infiltration, which prevent applications beyond thin tissues. Herein, we describe a two-step process to overcome these limitations. First, a dual electrospinning/electrospraying apparatus is used to incorporate cells into thin (~ 150 µm) meshes which are rolled. Second, the pores of the rolled meshes are filled with a cross-linked polyethylene glycol (PEG) hydrogel to create large cylinder-shaped composite meshes. In this study, the mechanical properties of these composite meshes – fabricated from both electrospun poly(lactic-co-glycolic acid) (PLGA) and poly(ester-urethane urea) (PEUUR) – are compared. Mechanical testing indicated that incorporation of the hydrogel does not change the overall mechanical properties. The PEUUR-based composite meshes have favorable mechanical properties for ligament applications due to their elasticity. Electrosprayed cells were visualized through histological cross-sections after 5 days of static culture, revealing nuclei distribution through the cross-section. Composite meshes were

cultured for 7 days under perfusion, cell number was maintained compared to immediately post-fabrication values and greater compared to static.

LOCATION AND DIRECTION DEPENDENT TENSILE PROPERTIES OF SWINE UTEROSACRAL AND CARDINAL LIGAMENTS. Ting Tan, Frances M. Davis, Suzanne Nicewonder & Raffaella De Vita, Department of Engineering Science and Mechanics, Virginia Tech, Blacksburg VA 24061. Uterosacral and cardinal ligaments help suspending the vagina and uterus on pelvic floor. Laxity of these ligaments can lead to women suffering pelvic organ prolapse (POP). Epidemiological studies suggest that the structural and mechanical changes occurring in these ligaments are critical to understanding the progression of POP. Specimens harvested from the uterosacral-cardinal ligament complex have been examined through scanning electron microscopy (SEM) and histology and tested for location and direction-dependent tensile mechanical properties. Collagen fibers were found to be arranged mainly in the longitudinal direction (normal to the long axis of vaginal wall) than the transversal direction (parallel to the long axis of vaginal wall). Sections of the ligaments stained with hematoxylin and eosin were also examined under microscope revealing smooth muscle orientation. The tensile testing results for uterosacral ligament, right cardinal ligament and left cardinal ligament specimens demonstrated that the mechanical response strongly depends on location. The tangent modulus of the linear region of the stress-strain curve varies significantly through the uterosacral-cardinal ligament complex, gradually increasing when specimens from cardinal ligaments transition into uterosacral ligaments. Moreover, the tensile testing findings on cardinal ligaments oriented in longitudinal and transversal directions indicated that these ligaments are anisotropic. Funding was provided by NSF CAREER Grant No. 1150397.

THE ROLE OF FIBRONECTIN SIGNALING IN EPITHELIAL TO MESENCHYMAL TRANSITION. Lauren A. Griggs, Dalton M. Berrie, Kirubel Feleke & Christopher A. Lemmon, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA 23284-3067. This work is part of an ongoing study that investigates the relationship between assembly of the extracellular matrix protein fibronectin and the occurrence of epithelial to mesenchymal transition (EMT). Previous studies have shown that a combination of growth factors, specifically Transforming Growth Factor-beta (TGF- $\beta$ ), and substrate stiffness drive epithelial cells to undergo EMT. Fibronectin's ability to serve as a growth factor delivery system along with its tendency to be assembled by cell-generated forces, which become larger on stiffer surfaces, led us to examine the hypothesis that substrate stiffness drives fibronectin assembly, which creates a high concentration of binding sites at the cell surface for various growth factors such as TGF- $\beta$  that in turn regulate EMT. Here we show that when MCF10A mammary epithelial cells were grown in the presence of TGF- $\beta$ , there was a reorganization of the mesenchymal marker actin into stress fibers, a decrease in the epithelial marker E-cadherin at the cell-cell junctions, and an increase in fibronectin assembly. To further define the role of fibronectin assembly in EMT, we demonstrated that in the presence of TGF- $\beta$ , EMT marker expression was significantly decreased when fibronectin assembly was inhibited by addition of the functional upstream domain (FUD) fibronectin-binding motif of the bacterial cell wall protein adhesin F1. (Supported by: an American Cancer Society Institutional Research Grant

via VCU/Massey Cancer Center and funds from the VCU Presidential Research Incentive Program).

EYE RESPONSE TO OVERPRESSURE FROM FIREWORKS. Vanessa D. Alphonse, Andrew R. Kemper, Brock T. Strom III, Stephanie M. Beeman & Stefan M. Duma, Virginia Tech-Wake Forest Univ. Center for Injury Biomechanics, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061. Injuries from fireworks are prevalent among youth. The eye is the most frequently injured body part and accounts for more than 2000 fireworks-related injuries annually. Although it is suggested overpressure can cause serious eye injuries, there is no empirical evidence to support this. The purpose of this research is to assess whether overpressure from fireworks can cause eye injury. This study evaluates the response of six human cadaver eyes to charges at distances of 22 cm, 12 cm, and 7 cm from the cornea. A pressure sensor inserted into the eye measured intraocular pressure. An array of pressure sensors around the eye was used to calculate rise time, positive duration, positive impulse, and wave velocity. High speed video was recorded for each event. No major eye injuries were observed; however, minor corneal abrasions were caused by projected material (confirmed with high speed video). Intraocular pressure was used to calculate injury risk, which was less than or equal to 0.01% for hyphema, lens damage, retinal damage, and globe rupture. The low calculated injury risk supports the lack of observed injuries. The combined presence of injuries caused by projected material and lack of injuries directly caused by overpressure indicated that overpressure from fireworks does not cause major eye injury at these energy levels.

MATERIAL DESIGN IN INSECT RESPIRATORY TISSUE. Matthew R. Webster & Raffaella De Vita, Dept. of Engineering Science and Mechanics, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. The respiratory system of insects is a natural microfluidic system. It is composed of a network of air filled tracheal tubes, many of which undergo periodic collapse and re-inflation during convective transport. The material design at multiple scales dictates the local behavior of the structures and their role in the transport of fluid. From a combination of mechanical testing and scanning electron microscopy (SEM) some aspects of the material design have been revealed. The results show that the trachea is orthotropic with very stiff thickenings known as taenidia lining the inner walls in the circumferential direction and providing compressive strength. Axially, a chitin fiber layer connects the taenidia and limits the axial extensibility. SEM micrographs revealed structural irregularities such as bifurcation in the taenidia in some locations. These irregularities were found to be more prevalent in the bending locations of the primary thoracic trachea indicating a possible role in the localized collapse of the tissue associated with convective respiration. Further tests will examine if differences in the taenidia organization correlate with differences in function of the trachea throughout the body. This study was supported the NSF-EFRI program grant No. 938047.

PIV INVESTIGATION OF ASCENDING AORTIC ARCH WITH A TILTING DISC VALVE. S. J. Warren, C. E. Taylor and G. E. Miller, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23285. For this project the object is to look into the effects of shear dependent viscosity on the flow

fields with the physiological. To isolate the parameters of the experiments the flow that is set to be investigated is the flow of blood out of the aortic valve into the aortic root. This is an area of flow with non-uniform shear rates which should have a pronounced effect on the velocity profile of a Newtonian versus non Newtonian fluid. This experiment has been completed before in literature to allow for comparison to data of previous works. The focus here is the comparison of shear dependent fluids and the fact that the fluid is tunable and controlled based on its computational model and the flow apparatus, the aorta, is made from open source patient-specific data that can be used to recreate that specific aorta. The valve for these experiments will be a tilting disc valve, which allows for known flow characteristics. The goal will be to conduct a statistical comparison that will give a non-dimensional score of the flow field to allow for a quantitative analysis of the device in the flow field.

### Botany

QUANTITATIVE ANALYSIS OF ANTIOXIDANTS IN SPECIALTY WINES. Alexandra C. Hcil, Hannah E. Wines, & Michael H. Renfroe, Dept. of Biol., James Madison University, Harrisonburg VA 22801. Wines are sources of antioxidants such as procyanidins and resveratrol along with polyphenolics that provide important health benefits and help protect against hypertension, arteriosclerosis, cancer, and other diseases. Most commercial wines are made from the domesticated European grape (*Vitis vinifera*). However, there are specialty wines that are made from native American grapes (*Vitis labrusca*), and wines that are fruit based or blended with fruit extracts. Our research compared the antioxidant levels found in muscadine and scuppernong wines to those found in fruit-based or fruit-flavored wines. Antioxidants were measured using the trolox equivalent antioxidant capacity assay (TEAC) and results were reported in  $\mu\text{mol}$  trolox equivalents (TE) per mL. Muscadine and scuppernong wines from a single vineyard were compared and antioxidant concentrations ranged from 2.2-5.5  $\mu\text{mol}$  TE/mL wine. Four fruit wines were compared from a single vineyard and the raspberry and peach were low in antioxidants (2.0 and 1.7  $\mu\text{mol}$  TE/mL, respectively), whereas the blackberry and chocolate-flavored wines were very high in antioxidants (10.7 and 12.6  $\mu\text{mol}$  TE/mL, respectively). Other fruit wines were tested and fell either within this range or slightly below it. These results indicate that wines can vary significantly in their antioxidant content, and that scuppernong and muscadine wines were comparable to some reported values of European grape-based wines.

QUANTITATIVE ANALYSIS OF ANTIOXIDANT ACTIVITIES IN TOMATOES AND PROCESSED TOMATO PRODUCTS. Anna T. Dinh & Michael H. Renfroe, Dept. of Biol., James Madison University, Harrisonburg VA 22801. The amount of antioxidants present in food varies depending on environmental conditions in which produce was grown and how products were processed prior to consumption. This study focused on quantitative analysis of antioxidant activities in commercially produced whole tomatoes and processed tomato products (paste, sauce, juice, diced, and soup) and fresh tomatoes grown experimentally in different light intensities. Variations and significant differences were observed between different types of whole tomatoes,



different types of processed tomatoes, different batches of the same brand and type of processed tomatoes. These variations could be attributed to the different locales tomatoes were grown, type of tomatoes used for processed tomato products, or additives such as herbs and spices used for flavoring. Data also indicated statistical differences between tomatoes grown under the same as well as different light intensities, on fw basis. Tomatoes grown in 100% light had TAA ranging from 1.898 to 3.565 mmol TE/g fw (7.73 to 13.405 mmol TE/g dw), 2.375 to 2.523 mmol TE/g fw (8.558 to 13.223 mmol TE/g dw) in 50% light, and 1.623 to 1.958 mmol TE/g fw (8.068 to 13.073 mmol TE/g dw) in 25% light. While small differences in data proved to be statistically significant, some of these differences may be too small to be of biological consequence. Data from this study, along with currently available data on antioxidants in foods, can provide useful information to consumers interested in purchasing products that are most beneficial to their health and to dietitians when making dietary recommendations to patients.

USING THE FLORISTIC QUALITY ASSESSMENT INDEX (FQAI) AT THE ABRAMS CREEK WETLANDS, A CALCAREOUS WETLAND COMPLEX IN WINCHESTER AND FREDERICK COUNTY VIRGINIA. Daniel F. Cooper & Woodward S. Bousquet, Environmental Studies Program, Shenandoah University, Winchester, VA 22601. The Abrams Creek Wetlands consist of more than 50 acres that are underlain by limestone bedrock and straddle the boundary between Winchester City and Frederick County, Virginia. The wetlands were divided into ten sites based on community types and property boundaries. Each site was visited twice during the 2013 growing season to record its vascular plant species. Unknown specimens were reviewed and identified by Gary Fleming of the Virginia Natural Heritage Program. A total of 227 species from 63 families were found and identified. Two species, *Symphytotrichum praealtum* var. *angustior* (Willowleaf Aster) and *Scutellaria galericulata* (Hooded Skullcap), have not been found at any other site in Virginia. After a final species list was compiled for each site, as well as the wetlands as a whole, floristic quality calculations were performed. Through the Floristic Quality Assessment Index (FQAI), the Abrams Creek Wetlands achieved a score of 51.9, which signifies high floristic quality. Scores calculated for each of the ten sites permit numerical, not subjective, evaluations of these different portions of the wetlands. The FQAI will allow researchers to assess the impacts of any future disturbances. Furthermore, this approach will permit managers of natural areas to set priorities for preservation and restoration.

RADIAL GROWTH DYNAMICS OF A RARE WHITE CEDAR (*THUJA OCCIDENTALIS* L.) FOREST IN THE SHENANDOAH VALLEY, VIRGINIA. Angela N. Felicio<sup>1</sup>, Joshua A. Kincaid<sup>2</sup>, & Cory M. Miller<sup>2</sup>, <sup>1</sup>Department of Biology, Shenandoah University and <sup>2</sup>Environmental Studies Program, Shenandoah University Winchester VA. 22601. Northern White Cedar (*Thuja occidentalis* L.) is a boreal coniferous tree species found from Quebec to Manitoba, Canada. Smaller disjunct populations occur southward along the Appalachian Mountains extending to Tennessee and western North Carolina. During the summers of 2011 and 2012 Shenandoah University researchers gathered samples by increment borer to document the growth-climate relationships of a disjunct Northern White Cedar forest in the Shenandoah

Valley. A total of 37 cores were collected and analyzed. Correlation analysis was used to determine the ring width response to climate from 1919-2012. There was a positive relationship with precipitation in the months of June and July ( $p < 0.05$ ). Discharge in the Shenandoah River was positively correlated from June-September ( $p < 0.05$ ). The Palmer drought severity index (PDSI) showed positive correlations during June-December and February-March ( $p < 0.05$ ). Given that only five stands of this species have been studied in Virginia, analysis of growth dynamics is instrumental in understanding this species.

*COMMELINA BENGHALENSIS*, NEW TO VIRGINIA. W. John Hayden, Department of Biology, University of Richmond, Richmond, VA 23173. *Commelina benghalensis* L. (Benghal dayflower) was discovered growing spontaneously among ornamental landscape plants on the University of Richmond campus on 19 October 2012. This federally listed noxious weed can be distinguished from other species of *Commelina* found in Virginia by the combination of spathes bearing chasmogamous flowers at aerial stem tips and nearly closed spathes bearing cleistogamous flowers from proximal, often subterranean, lateral branches. The population consisted of three mature plants bearing numerous aerial and subterranean spathes and nine non-reproductive seedlings. All individuals found were removed and used to make specimens for anatomical study and herbarium vouchers; vouchers will be distributed from URV to other major herbaria in Virginia. Searches of other landscape plantings in the vicinity failed to reveal additional occurrences of the plant. Benghal dayflower has spread progressively northward from its discovery in Florida in 1928. Given the setting of the population discovered, these plants may have been dispersed coincidentally with landscape plants. This is the first record of *Commelina benghalensis* to occur in Virginia where its status should be considered a non-native waif.

THE FLORA OF VIRGINIA HAS BEEN PUBLISHED: THE FUTURE OF THE FOUNDATION OF THE FLORA OF VIRGINIA PROJECT. Marion B. Lobstein, Professor Emeritus, Northern Virginia Community College, Manassas, VA 20109. Virginia, for its landmass, has the most diversity of vascular plant species of any state in the United States. The Colony of Virginia had the first colonial flora, *Flora Virginica*, which was last updated in 1762. Since 1926, the Virginia Academy of Science has supported efforts to produce a modern *Flora of Virginia*. The Foundation of the *Flora of Virginia*, Inc, was formed in 2001 to realize this goal. The *Flora of Virginia* was published in December 2012. The Foundation of the Flora of Virginia project will continue to function to accomplish future goal of the Project. One of those goals is the development of teaching and learning modules. Marion Lobstein shared details of her efforts to develop workshops and materials to facilitate the use of the new *Flora of Virginia* by interested adult groups. Botany section members were also encouraged to share their ideas to begin to implement this goal.

THE FLORA OF VIRGINIA, A MANUAL TO THE MANUAL AND THE FUTURE OF THE PROJECT. J. Christopher Ludwig, Virginia Department of Conservation and Recreation, Division of Natural Heritage, Richmond, VA 23102. The 1554-page *Flora of Virginia* was published in November, 2012. This manual to the vascular plants of

Virginia treats 3164 native and naturalized taxa. Many conventions and shortcuts that are used in the Flora of Virginia will be presented which will make the manual easier and more useful to use. All information within the Flora of Virginia has been maintained in a database for eventual digital presentation in combination with other databases that have additional information on the distribution and habitats of Virginia plants. This digital presentation will likely take the form of an app for handheld devices. The app will be designed for use in the field and will link to an image library. These and other future plans will be detailed.

EVOLUTIONARY HISTORY OF CANARY GRASSES: PHYLOGEOGRAPHY, CYTOLOGY AND FLORET STRUCTURE. Stephanie M. Voshell & Khidir W. Hilu. Dept. of Biological Sciences, Virginia Tech, Blacksburg, VA. *Phalaris* (Poaceae) contains 21 endemic to cosmopolitan species, found throughout the temperate and subtropical regions of the world with distinct centers of diversity in the Mediterranean Basin and western North America. The species display annual or perennial habit and possess basic chromosome numbers of  $x=6$  and 7, with polyploidy confined to the later cytotype. *Phalaris* presents a valuable opportunity to study long distance dispersal, historic hybridization and polyploidy events, and the impact of floret structure on natural range expansion in grasses. We present here the first phylogeographic study showing the historic events of migration, speciation and dates of divergence in the genus using Bayesian inference and dispersal-vicariance analyses. The genus emerged in the Mediterranean Basin during the Miocene epoch (23-5.3 MYA). A basal split occurred between the  $x=6$  and  $x=7$ , with the former group (3 species) remaining as diploids in the Mediterranean region and the later group expanding into Africa and Asia and evolving various ploidy levels. Dispersal to North America via the Bering Land Bridge resulted in diversification in the Americas based on diploid speciation. The greater speciation and dispersal success of the diploid  $x=7$  cytotype compared to its  $x=6$  counterpart raises questions about the significance of this early chromosome rearrangement. Florets with prominent and pubescent sterile lemmas, as seen in the New World species, may be advantageous for dispersal via animal transport.

GENES VS. GENOMES IN RESOLVING DIMENSIONALITY OF THE TREE OF LIFE: CARYOPHYLLALES AS A CASE STUDY. Khidir W. Hilu & Sunny S. Crawley, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. Decline in sequencing costs resulted in a surge in the number of organelle genome sequences, providing an abundance of molecular characters for molecular phylogenetics. Phylogenetic reconstruction based on whole genome matrices although tending to provide robust trees, is generally strongly skewed toward relatively narrow taxon representation. In contrast, datasets based on a few genomic regions although promoting a denser taxon sampling, are construed as potentially too character-poor to accurately resolve phylogenetic histories. Using the angiosperm order Caryophyllales as a taxonomic platform, we contrast the relative efficacy in phylogenetic reconstruction of whole genome/narrow taxon representation vs. few genomic regions/denser taxon sampling, and explore the potential of combining these two types of datasets, allowing for the inherently large proportion of missing data. Our preliminary results demonstrate that a prudent selection of a limited number of genomic regions can provide a phylogenetic tree that approaches the genome-based tree with the

added benefit of considerable detail at recent histories. Combining the dataset recovered the backbone and provided valuable information on the terminal nodes despite extensive amounts of missing characters/genomic regions. This approach provides a cost- and time-effective platform for discerning the dimensionality of the tree of life.

#### Posters

INVESTIGATING THE ROLE OF STP1 IN OSMOTIC BALANCE IN ARABIDOPSIS THALIANA. Janet C. Daniel, Morgan Donovan, Ji Lee, Tiffanie Le & Maria Wilkins, Department of Biology, James Madison University Harrisonburg, VA. 22807. *Arabidopsis thaliana* is a commonly used model organism in plant research and is described as a glycophytic plant. In this study, we investigated the role of sugar transport protein-1 (STP1) in salt resistant phenotype exhibited by the STP1 k/o plant by observing WT and STP1 k/o plants watered with various concentrations of NaCl. We measured plants grown in soil, using a hydroponics system and on plates. RESULTS: STP1 k/o roots grown in soil and watered with 50mM NaCl solution are significantly longer than WT plants at 2 weeks (12.1 mm vs. 6.03 mm,  $p=2.4 \times 10^{-3}$ ). On average, STP1 roots (16.62 mm vs. 9.93 mm at 3 weeks) and shoot growth (3.4 mm vs. 2.77 mm at 2 weeks,  $p=1.29 \times 10^{-4}$ ) in 50 mM NaCl were longer than than WT. In order to obtain increased plant tissue to initiate gene expression studies, we have adapted a hydroponics system to grow plants in larger quantities for ease in harvesting tissues for molecular and microscopic analysis. Growing plants hydroponically results in comparable root and shoot growth to growth on soil and agar plates. WT roots grown in 50mM NaCl were significantly different than those grown in 0mM NaCl (13.07 mm vs. 5.67 mm,  $p=5.1 \times 10^{-3}$ ) while STP1 k/o were not significantly different (11.89 mm vs. 9.67 mm,  $p=0.26$ ). Characterization of the mechanism of salt tolerance exhibited by the *Arabidopsis thaliana* STP1 knockout will provide further insight into role STP1 plays in osmotic regulation of the plant.

PROGRESS IN PHYLOGEOGRAPHIC STUDY OF NORTH AMERICAN MELAMPYRUM LINEARE (OROBANCHACEAE). Karoline Oldham & Andrea Weeks, School of Systems Biology, George Mason University, Fairfax VA 22030 & Dept. of Environmental Science and Policy, George Mason University, Fairfax VA 22030. *Melampyrum lineare* is the sole North American species of its genus, which is otherwise distributed in Europe and Asia. It is not currently known how this species migrated to North America. Current literature completely omits *M. lineare* from the taxa sampled for genus- and family-level phylogenetic studies. As a consequence, this species' relation to other members of *Melampyrum* is poorly understood. Morphology suggests a European progenitor, *M. pratense*, which implies ancestral migration across the Atlantic Ocean. However, this hypothesis has not been thoroughly investigated. Comparison of ITS sequences of *M. lineare* with 11 other species of *Melampyrum* and 21 other closely related taxa does not support a close relationship between *M. lineare* and *M. pratense*. Rather, the bootstrap 50% majority-rule consensus tree provides minimal support for a sister grouping of *M. lineare* with two Asian species, *M. klebelsbergianum* and *M. roseum*, while the 50% majority-rule consensus tree places *M. lineare* as sister to a large clade containing *M. pratense*, with *M. klebelsbergianum*

and *M. roseum* sister to this grouping. If *M. lineare* is indeed descended from Asian species of *Melampyrum*, this implies a drastically different migration history than previously hypothesized. Inclusion of another widely distributed Asian species, *M. laxum*, and phylogenetic analysis of other markers will further elucidate the relationships between *M. lineare* and other members of *Melampyrum*.

A PRELIMINARY CHECKLIST OF THE FRESH-WATER PLANKTONIC MICROALGAE OF THE NORTH LAGOON AT CROOKED TREE, BELIZE, C. A. Stephen W. Fuller, Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA 22401. Tucked in on the south end of the Yucatan peninsula, on the east side, is the small country of Belize. The northern half of the country consists of low, flat habitats, generally not exceeding 330 feet in elevation, and including lagoons, and flat plains. The lagoons vary tremendously in volume, and hence water level, with the seasonal change from the rainy to the dry season. The fresh-water algal plankton of Belize is relatively unknown, with only two studies, in 2003 and '04, reporting on plankton from two lagoons in southern Belize. In March of 2013 the North Lagoon at Crooked Tree Wildlife Sanctuary was sampled, and 31 taxa were identified. The sample was dominated by Desmids, the Chlorophyta having the most diverse representation at 15 taxa. Species of *Pediastrum* and members of the Cyanobacteria were also prevalent. But frequently, specimens of *Synura*, *Scenedesmus*, and *Dinobryon* were observed, and less frequently, specimens of diatoms and *Euglena*. A thorough investigation of the fresh-water phytoplankton throughout the country and the seasons would provide an interesting exposition of this part of the community.

A PHYLOGENETIC TREE OF THE PEANUT GENUS *ARACHIS* (FABACEAE) BASED ON *TRNT-F/ITS* VS. ALLERGEN GENES. Jenna Sackenheim, Sheena Friend, Chandra Shrestha & Khidir Hilu, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. The legume genus *Arachis* L. (Fabaceae) contains 80 annual and perennial, diploid and polyploid species, including the economically important crop peanut (*Arachis hypogaea*). We are exploring the usefulness of the nuclear allergen genes in *Arachis* phylogenetics. Sequence information from two allergen orthologs Ara h 2 and Ara h 6 for 17 species of *Arachis* were analyzed with RAxML to assess the phylogenetic relationships among the species. The tree based on Ara h 2 was highly congruent with the ITS/*trnT*-F tree obtained by Friend et al. (2010). In contrast, the Ara h 6 tree, although resolving some of those relationships, failed to discern others. This shortcoming might be due to either differences in amount of phylogenetic signals or varying selection pressures operating on the orthologs. Our future study will focus on addressing the issues stated above.

## Chemistry

PROGRESS TOWARDS THE SYNTHESIS OF DRAGOMABIN. Michelle K. Waddell, Dept. of Chem., Hampton University, Hampton, VA 23668. Malaria is a degenerative disease caused by a parasitic infection transmitted by infected female *Anopheles* mosquitoes. Worldwide 350-500 million cases have been reported with an attributed 1 million deaths. Currently, Artemisinin is the last line of defense against

Chloroquine-resistant malaria parasites. Due to the pressure on governments to switch to more effective treatments, it is conceivable that Artemisinin-resistant parasites are on the horizon. There has been an increased interest in developing new drugs due to the danger of transmission of anti-malarial resistance in malaria parasites. Dragomabin is one of a class of lipopeptides with anti-malarial activity isolated from a Panamanian strain of the marine cyanobacterium *Lyngbya majuscula*. While it is known that Dragomabin can bypass a cellular membrane, the mechanism of incorporation is unknown. The lipopeptides Carmabin A, Dragomabin, and Dragonamide A have  $IC_{50}$  = 4.3, 6.0 and 7.7  $\mu$ M respectively. The goal of this research is to perform the first synthesis of Dragomabin in its enantiomeric pure form. The aliphatic side chain will be synthesized in solution phase. Standard Wang solid phase protocol will be used to synthesize the tetrapeptide. Results of the synthetic strategy are presented. Once the synthesis has been completed, structural analogs will be constructed by varying the substituents on the core molecule. These analogs will be used to determine the structure activity relationships of these molecules in order to increase their biological activity against Chloroquine-resistant *Plasmodium falciparum*.

EXPLORING THE COORDINATION CHEMISTRY OF CURCUMINOIDS: SYNTHESIS AND BIOPHYSICAL REACTIVITY. Floyd A. Beckford, Shylene A. Scott, Kinsey L. Hall & Samantha Smith, Dept. of Natural Sciences University Virginia College at Wise, Wisc, VA 24293. Curcuminoids are diarylheptanoids which contain a  $\beta$ -diketone group and are analogs of curcumin an important polyphenol long-known for its biological activity. These molecules exhibit many therapeutic properties being applied as antioxidant, anticancer preventive, antibacterial and anti-inflammatory agents. Despite the fact that the diketone moiety can act as a chelating site the coordination chemistry of curcuminoids have not been extensively studied. Recent research into ruthenium-based organometallics suggests potential medicinal use due to ruthenium's low toxicity to healthy cells and good selectivity for malignant cells when compared to competing metals. In this paper a set of structurally diverse curcuminoids were synthesized by the reaction of 2,5-pentanedione with the appropriate aldehyde in the presence of tributylborate and *n*-butylamine as catalyst. These curcuminoids were used as mono-anionic bidentate ligands in the synthesis of half-sandwich organometallic ruthenium complexes of the type  $[(\eta^6\text{-arene})\text{Ru}(\text{curcuminoid})\text{Cl}]$  where arene = benzene or *p*-cymene. The complexes have been characterized and initial investigations of reactions with DNA as well as some model proteins have been carried out. A number of the complexes show a weak ability to uncoil pBR322 plasmid DNA but this ability disappears under irradiation with 365 nm UV light. Spectroscopic and viscometric experiments suggest that the complexes interact with DNA via weak intercalation. On the other hand the interaction, as measured by the binding constant, with human serum albumin is strong.

INVESTIGATION OF COPPER RUNOFF IN SOIL SAMPLES. Darren Driscoll & M.E. Howard, Dept. of Chem., Virginia Wesleyan College, Norfolk, VA, 23502. With rain being naturally acidic and with the continuance of acid rain throughout industrial areas; the leaching of metals into the water and soil of industrial areas is an important topic. This research was geared towards looking at how copper siding would be affected by this acidified rain on a small scale. Copper siding or cladding is used to

cover two external walls on Clark Hall, one of the buildings on the campus of Virginia Wesleyan College. The objective of this research was to develop a method to investigate the effects of rain on the copper building material. This was done by determining if copper concentrations were greater in areas of high probability for copper runoff and by investigating whether the copper runoff had high mobility through the soil, possibly impacting the groundwater systems. An exchangeable ion method of extraction was used and the solutions were analyzed using Flame Atomic Absorption Spectrometry (FAAS). It was found that soil concentrations of copper were significantly higher in areas of high copper runoff probability (adjacent to the wall with copper siding) compared to control locations. The highest value of copper near the build was found to be  $31.8 \pm 0.5 \mu\text{g} / \text{g}$  of soil. It was also found that the copper runoff had a little mobility through the soil which suggested that the copper is accumulating in the soil underneath the building.

**SUBSTITUTION VERSUS ELIMINATION: THE INFLUENCE OF THE LEAVING GROUP.** Charles M. Bump, Dept. of Chem., Hampton University, Hampton, VA 23668. A survey of the competition between  $\text{S}_{\text{N}}2$  and E2 reactions is an important part of the study of mechanistic organic chemistry. Generalizations are presented with little theoretical background in support of those statements. We present a review of the calculated (DFT-B3LYP – 3-21G) thermodynamics of different leaving groups as a factor in determining the favored product in the reaction between sodium hydroxide and an ethyl group bonded to a leaving group. We considered the halides (F, Cl, Br, I), acetate, tosylate, hydroxide ion, and methoxide ion as leaving groups. The favored pathway ( $\text{S}_{\text{N}}2$  or E2) appears to be independent of leaving group. All substrates share a common “crossover temperature” above which elimination is favored and below which substitution is favored.

**BORON-ACRIDINE CHIMERAS: FUNCTIONALIZING BRANCHED PEPTIDES TOWARD TARGETING HIV-1 RRE RNA.** Jessica Wynn, Wenyu Zhang & Webster L. Santos, Dept. of Chem., Va. Polytechnic Inst. and State Univ., Blacksburg, VA 24061. While human immunodeficiency virus type 1 (HIV-1) is manageable with current antiretroviral therapies, the majority of FDA approved drugs for HIV-1 target enzymes that are prone to high rates of mutation, such as HIV-1 reverse transcriptase. This leads to drug resistance over time, creating the need for therapeutic alternatives. Our research focuses on targeting a conserved region of the HIV-1 genome, the rev response element (RRE). Branched peptides are utilized as a scaffold for multivalent binding to RRE IIB with the incorporation of boronic acid side chains to increase binding affinity via possible interaction with the 2'-OH group of RNA. A high-throughput screening assay of a 3.3.4-branched peptide boronic acid library revealed a hit compound that selectively bound to RRE IIB RNA in the submicromolar range, and was both cell permeable and non-toxic up to  $30 \mu\text{M}$ . To further improve the binding affinities of our peptides, acridine was incorporated into a new 3.3.4-branched peptide boronic acid library. Our findings indicate that a hit compound containing boronic acid and acridine resulted in an improved binding affinity in the low nanomolar range.

DEVELOPMENT OF SPHINGOSINE KINASE II SELECTIVE INHIBITORS: DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY. Molly D. Congdon<sup>1</sup>, Neeraj Patwardhan<sup>1</sup>, James Gumkowski<sup>1</sup>, Elizabeth Childress<sup>1</sup>, Emily Morris<sup>1</sup>, Yugesh Kharel<sup>2</sup>, Kevin Lynch<sup>2</sup> & Webster L. Santos<sup>1</sup>, <sup>1</sup>Dept. of Chemistry, Virginia Tech, Blacksburg, VA 24061 & <sup>2</sup>Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22908. Sphingosine-1-Phosphate (S1P) is formed naturally through the phosphorylation of sphingosine (Sph) by the two isoforms of Sphingosine Kinase (SphK1 and SphK2). Increased concentrations of S1P have been implicated in a variety of diseases including cancer, fibrosis, multiple sclerosis and diabetes through intracellular and intercellular targets. SphK1 and 2 differ in size, cellular localization, and possess different intercellular roles. The highly studied SphK1 has been shown to be proliferative while the less known SphK2 has been shown to be pro-apoptotic. Selective inhibitors could aid in the understanding the physiological roles of these kinases. While a wide variety of SphK1 selective inhibitors have been synthesized, there exists an absence of high affinity, SphK2 selective inhibitors. This presentation will discuss the continuation of a tail region structure-activity-relationship based upon a novel SphK2 selective scaffold, SLR080811.

STRUCTURE ACTIVITY RELATIONSHIP STUDIES OF NOVEL GUANIDINE BASED INHIBITORS OF SPHINGOSINE KINASE-2. Neeraj N. Patwardhan<sup>1</sup>, Mithun R. Raje<sup>1</sup>, Emily A. Morris<sup>1</sup>, Kenneth Knott<sup>1</sup>, Yugesh Kharel<sup>2</sup>, Ming Gao<sup>1</sup>, Kevin Lynch<sup>2</sup> & Webster L. Santos<sup>1</sup>, <sup>1</sup>Dept. of Chemistry, Virginia Tech, Blacksburg, Virginia 24061 & <sup>2</sup>Dept. of Pharmacology, University of Virginia, Charlottesville, Virginia 22908. Sphingosine kinase (SphK) has emerged as an attractive target for various therapeutics due to its prominent role in processes such as cell proliferation, apoptosis etc. EN.CITEEN.CITE.DATA<sup>1</sup> SphK exists in two isoforms: SphK1 is localized in the cytosol while SphK2 is localized in the nucleus. These enzymes phosphorylate sphingosine to sphingosine-1-phosphate, which has been shown to signal intracellularly via HDACs and BACE1., and extracellularly via interactions with the five G-protein coupled receptors S1P<sub>1-5</sub>. This signaling pathway has recently been associated with a variety of different diseases. Recently, the Santos group has developed a novel guanidine based lead compound SLR080811 that selectively targets SphK2 with good selectivity and potency at a low micromolar  $K_i$ . The structural scaffold contains three regions that could be diversified into a variety of derivatives to improve selectivity: the head, linker and tail regions. In this presentation, we will highlight our current efforts towards developing different head-group analogs of SLR080811. The *in vitro* and *in vivo* activity of these inhibitors will be discussed.

BIOPHYSICAL ANALYSIS OF THE ACTIVE SITE LOOP OF BACTERIAL BETA-GLUCURONIDASE. Suzanne Hengeli & Kimberly Lane, Dept. of Chemistry, Radford University, Radford, VA 24142.  $\beta$ -glucuronidase has been associated with the side effects related to the cancer treatment drug CPT-11 (irinotecan hydrochloride), which is primarily used for colon cancer treatment. CPT-11 is converted in the body to the active form of the drug, SN-38, which is further metabolized into SN-38G (by addition of a glucuronide group). SN-38G is transported to the intestine for elimination, where it is hydrolyzed to SN-38 by bacterial  $\beta$ -glucuronidase. This release of SN-38 causes damage to the intestinal lining, leading to side effects such as severe



diarrhea. Recent studies have produced an inhibitor that is specific for the bacterial form of  $\beta$ -glucuronidase and have demonstrated that this specificity is due to the presence of an active site loop found in the bacterial form of the enzyme, but not the human form. In this study, point mutations in the residues found in the active site loop of the enzyme, which are residues 360 through 376, were modeled using Win-Coot and Argus Lab. Residues in the active site loop were mutated to see how the binding energies would be affected, the tighter the binding energies the estimated better the inhibitor would bind to that active site.

THERMAL DECOMPOSITION OF METAL OXALATES. T. C. DeVore, Dept. of Chemistry and Biochemistry, MSC 4501, James Madison University, Harrisonburg, VA 22807. Although the dynamics of thermal decomposition of metal oxalates have been investigated for well over a century and hundreds of papers have now been published on the topic, the mechanism for this decomposition has not been established with certainty. DFT-B3LYP calculations using the 6-311G++ (3pd, 3df) basis set have been used to investigate the mechanism proposed by Boldyrev. The first step of this mechanism is the splitting of the C-C bond in the oxalate anion to form two  $\text{CO}_2^-$  anions. The activation energy calculated for this process (178 kJ/mol) agrees well with experimental activation energies measured for many transition metal oxalates. The  $\text{CO}_2^-$  anions can rearrange and form a stable intermediate ( $\text{CO}_3\text{CO}_2^-$ ) that decomposes to produce the observed products. The activation energy for the production of CO was calculated to be 94 kJ/mol. Molecular geometries and vibration frequencies for each intermediate have also been determined.

PSEUDOROTAXANES AS PRECURSORS OF MECHANICALLY LINKED ASSEMBLIES. Harry W. Gibson, Zhenbin Niu, Minjae Lee, Terry L. Price, Jr., Mason A. Rouser, Arun Murugan, Hanlie Wessels, Daniel V. Schoonover & Carla Slebodnick, Dept. of Chem. & Macromolecules & Interfaces Institute, Virginia Tech, Blacksburg, VA 24061-0212. Over the past 25 years we have utilized pseudorotaxane and rotaxane self-assembly to control the structures and properties of macromolecules. Suitable bisphenylene crown ethers, such as bis(*p*-phenylene)-34-crown-10 and bis(*m*-phenylene)-32-crown-10, complex 4,4'-bipyridinium compounds ("paraquats" or "viologens") have association constants,  $K_a$ , of 500 to 1000  $\text{M}^{-1}$ . Our use of these systems as models for construction of polymeric pseudorotaxanes and rotaxanes of various architectures will be reviewed briefly. Efficient formation of pseudorotaxanes for supramolecular polymerization and formation of block, graft and star polymers from cyclic host and guest components requires high association constants ( $K_a > 10^4 \text{ M}^{-1}$ ). We have prepared cryptands with association constants in the range of  $10^4$  to  $10^6 \text{ M}^{-1}$  with 4,4'-bipyridinium compounds ("paraquats" or "viologens") and have developed several efficient ways to functionalize the most powerful versions of these hosts, the pyridino cryptands. These functionalized hosts now enable new avenues to create supramolecular polymeric structures beginning with both functionalized polymers as well as properly functionalized monomeric species. Combined with modern polymerization methodologies these systems enabled our recent work directed toward formation of star, block, graft and brush copolymers and also supramolecular polymers. These procedures will be discussed along with characterization of the resultant novel materials.

DESIGN AND SYNTHESIS OF ROMP IMIDAZOLIUM POLYMERS FOR USE AS ACTUATORS. T. L. Price Jr.<sup>1</sup>, U. H. Choi<sup>2</sup>, D. Wang<sup>3</sup>, Arunachalam Murugan<sup>1</sup>, D. V. Schoonover<sup>1</sup>, M. Zhang<sup>1</sup>, R. H. Colby<sup>2</sup>, J. R. Heflin<sup>3</sup>, R. B. Moore<sup>1</sup> & H. W. Gibson<sup>1</sup>, <sup>1</sup>Dept. of Chem., Virginia Tech, Blacksburg, VA, 24061, <sup>2</sup>Dept. of Materials Science & Engineering, Penn State University, University Park, PA, 16802, & <sup>3</sup>Dept. of Phys., Virginia Tech, Blacksburg, VA, 24061. Previous work conducted by our group has established that properly designed poly(meth)acrylates containing imidazolium pendant groups will exhibit high single ion conductivities ( $\sim 10^5$  S/cm). Polymers of this type are known to be capable of electromechanical actuation. In this presentation we will describe our design criteria for poly(norbornene)s containing imidazolium pendant units and discuss advantages of the system; additionally, single ion conductivities ( $\sim 10^5$  S/cm) and preliminary studies of their actuation behavior will be presented. (Supported by: U.S. Army Research Office grant number W911NF-07-1-0452: Ionic Liquids in Electro-Active Devices (ILEAD) MURI).

DESIGN OF MULTICHROMOPHORIC SYSTEMS FOR MOLECULAR ELECTRONICS. E. M. N. Ndip, N. Pandey & K. Cole, Dept. of Chem. Hampton University, Hampton, VA 23668. This work is part of our ongoing research on the design, synthesis, and laser spectroscopy of multichromophoric (aryl styryl heterocyclic (thiophene, furan, and pyrrole)  $\pi$ -centered compounds) systems for molecular electronics. Theoretical studies have elucidated the effects of tuning on several electronic properties and determined their suitability as materials for molecular electronic devices. Ground state geometries of various derivatives were optimized using both semi empirical (PM3) and DFT-B3LYP with the 6-31G\*\* basis set. The linear absorption spectra, and energy gaps were computed for the various molecular systems in vacuum at both the semi empirical (ZINDO-CI) and *ab initio* (TDDFT) methods on the optimized ground state geometries. The computed maximum wavelengths for representative systems are in good agreement with the experimental data (405-438nm) obtained from fluorescence experiments cited in the literature. The data also shows reasonable agreement between the semi-empirical and *ab initio* calculated  $\lambda$ . Band gaps determined from the TDDFT/B3LYP calculations are more consistent with the experimental values for the thiophene derivatives. (Supported by NSF CREST (HRD-0734635, HRD-0630372, and ESI-0426328/002))

ELECTROCHEMICAL INTERACTIONS OF ACTIVATED CARBONS WITH AQUEOUS AND BIOLOGICAL MEDIA: AN ACID-BASE MODEL. M. M. Goldin<sup>1</sup>, G. R. Garacva<sup>2</sup>, & N. V. Sklifosovky<sup>2</sup>, <sup>1</sup>Dept. of Biol. & Chem., Liberty University, Lynchburg, VA 24502 & <sup>2</sup>Institute for Emergency Medicine. The adsorption of metal ions and organic compounds on activated carbons was measured in 0.10 M Na<sub>2</sub>SO<sub>4</sub> against Ag/AgCl. Activated carbon samples caused a drift in the pH of distilled water, 0.10 M sodium sulfate, and other solutions in which they were immersed. For carbons with positive OCP, the pH drifted toward acidic values, while for negative-OCP carbons, toward basic values. If the carbon/electrolyte heterogeneous equilibrium is considered as an Usanovich acid-base pair, the electron acceptor is an acid, and the electron donor is a base. The "acidic" positively charged carbon immersed in a solution acidifies it, while the "basic" negatively charged carbon makes it more basic, which is in agreement with empirical data. Identical unmodified AKU and AG-3

activated carbon samples were immersed in phosphate buffer solutions; the acidic solution caused a positive shift in the OCP of carbon, while the basic solution produced a negative OCP shift, also consistent with the Usanovich model. Finally, unmodified and oxidized SKT-6A carbon, as well as an iodide-doped polypyrrole (PPy) composite based on SKT-6A and pure chloride-doped PPy, were titrated by the Boehm method to determine surface functionalization. The amount of carboxyl groups on the surface of these materials gives a strong linear correlation with OCP and overall functional group data corroborates the increase in Brønsted-Lowry acidic groups on carbon surface with increasing potential. This study is supported by funds from the Center for Research and Scholarship Fund of Liberty University.

$\eta^2$ -DEAROMATIZATION: MODIFICATIONS OF ANILINE AND 2-(DIMETHYLAMINO) PYRIDINE. J. A. Pienkos<sup>1</sup>, A. Knisely<sup>1</sup>, B. Liebov<sup>1</sup>, V. Teran<sup>1</sup>, V. E. Zottig<sup>1</sup>, M. Sabat<sup>2</sup> & W. H. Myers<sup>3</sup>, <sup>1</sup>Dept. of Chem., University of Virginia, Charlottesville VA 22903, <sup>2</sup>Nanoscale Materials Characterization Facility, Dept. of Materials Science and Engineering, University of Virginia, Charlottesville VA 22903, & <sup>3</sup>Dept. of Chem., University of Richmond, Richmond VA 23173. Organic scaffolding with a transition metal fragment allows for rapid access to new compounds.  $\eta^2$ -coordination of an arene to a  $\pi$ -basic {TpW(NO)(PMe<sub>3</sub>)} fragment renders the aromatic molecule “dearomatized.” A dearomatized arene exhibits reactivity that is different than the reactivity of the free, unbound ligand. Ligand exchange between {TpW(NO)(PMe<sub>3</sub>)}( $\eta^2$ -benzene) and 2-(dimethylamino) pyridine or *N,N*-dimethyl aniline derivatives, in the presence of acid, affords new  $\eta^2$ -complexed cationic species. These species can react with various electrophiles, generating allyls, which can react with aromatic molecules to form a carbon-carbon sp<sup>3</sup>-sp<sup>2</sup> center. Additionally, the  $\eta^2$ -coordinated *N,N*-dimethyl aniline derivative can be cyclopropanated and further modified through ring opening reactions. Liberation of the bound ligands affords novel  $\alpha,\beta$ -unsaturated cyclohexenones.

STEP: SOLAR THERMAL ELECTROCHEMICAL PROCESS, A COMPREHENSIVE PROCESS FOR UTILIZING RENEWABLE ENERGY TO REDUCE ANTHROPOGENIC CARBON DIOXIDE EMISSIONS. Stuart Licht, Baochen Cui, Baohui Wang, Jason Lau & Jessica Stuart, Dept. of Chem., The George Washington Univ., Washington, DC. 20052. STEP is a new process that converts and stores solar energy. STEP has been shown experimentally to function at much higher efficiencies than photovoltaic solar cells and to support current densities that are 1 to 3 orders of magnitude greater than those achieved by photoelectrochemical water splitting or photoelectrochemical solar cells. STEP utilizes a portion of sunlight to drive photovoltaic charge transfer, while the remainder of the sunlight is applied as solar thermal energy to heat and decrease the energy of endothermic electrolysis reactions. By taking the reactants to a higher temperature, the voltage needed to drive the endothermic electrolysis reaction decreases improving the efficiency of the reaction. A secondary advantage of going to these higher temperatures is the ability to use molten salts as the medium for electrolysis. Molten salts can support very high molecular concentrations at the electrode surface allowing for higher current densities with greater efficiencies. Select molten salts can support very high voltages, are resistant to poisoning and can act as a thermal buffer to maintain the high temperature

needed for the reaction. STEP has been shown to be a suitable alternative for a variety of carbon dioxide releasing processes such as the production of iron, cement, hydrogen, desalinization, fuels, and wastewater treatment giving STEP the potential to be a comprehensive approach for reducing anthropogenic carbon dioxide emissions.

#### Posters

CHEMO- AND REGIOSELECTIVE DIBORATION OF ALLENES. Xi Guo, Amanda Nelson & Webster L. Santos, Dept. of Chem., Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061. Organoboron compounds are important synthetic intermediates in organic synthesis. Metal-catalyzed diboration reaction with commercially available diboron reagents is a traditional method to introduce boron moiety. However, most of the previous work was focused on symmetrical diboron reagents such as bis(pinacolato)diboron and bis(catecholato)diboron, a differentially protected diboron reagent is employed in this work. Diboration of different allenes with it provides the borylated product chemo- and regioselectively. This work is funded by Department of Chemistry, Virginia Polytechnic Institute and State University and ACS Petroleum Research Fund.

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL INHIBITORS OF SPHINGOSINE KINASE 2. Emily A. Morris<sup>1</sup>, Neeraj N. Patwardhan<sup>1</sup>, Mithun R. Raju<sup>1</sup>, Yugesh Kharol<sup>2</sup>, Kevin Lynch<sup>2</sup> & Webster L. Santos<sup>1</sup>, <sup>1</sup>Dept. of Chem., Virginia Tech, Blacksburg, Virginia 24061 & <sup>2</sup>Dept. of Pharmacology, University of Virginia, Charlottesville, Virginia 22908. Sphingosine kinase (SphK) has become a prevalent target for many disease states in recent years. SphK phosphorylates sphingosine to sphingosine 1-phosphate (S1P). SphK can be found in two isoforms, SphK1 and SphK2, and differ in their cellular location. In mouse studies, inhibition of SphK2 showed an increase in the circulating levels of S1P in blood. This increase of S1P has been shown to be anti-fibrotic, as it leads to a decrease in the extracellular-matrix formation. Our lead molecule, SLR080811, is shown to have a  $K_i$  of 1  $\mu$ M and 5-fold selectivity for SphK2 over SphK1. SLR080811's structural motif contains a tail group, linker, and head group. In this poster, we will review the structural modifications that have been performed to the linker and head group regions of SLR080811 in an attempt to improve inhibition of SphK2.

MUTAGENIC ANALYSIS OF BACTERIAL BETA-GLUCURONIDASE. Skye Hickling, Gina Burchett & Kimberly Lane, Dept. of Chem., Radford University, Radford, VA 24142. Camptothecin-11 (CPT-11) is used for chemotherapy in cancer patients. It gets converted to the topoisomerase inhibitor SN-38. SN-38 is metabolized in the liver to SN-38-glucuronide. Bacterial  $\beta$ -glucuronidase hydrolyzes the glucuronide re-releasing SN-38 into the intestines. This reformed SN-38 is toxic to the body and  $\beta$ -glucuronidase is the key to the toxicity of the drug CPT-11. The use of inhibitors of *E. coli*  $\beta$ -glucuronidase decreases the amount of damage to intestinal cells.

Deficiencies in the human form of  $\beta$ -glucuronidase may lead to the onset of Sly syndrome, a lethal disease with no current treatment. Previous studies has shown that a stable dimer of the enzyme may exist. This current study has been using WinCoot

and ArgusLab to develop mutations *in silico* of the active site in  $\beta$ -glucuronidase and subunit interfaces. Active site mutations were performed at residues that have shown to be of some importance through the enzyme related to  $\beta$ -glucuronidase called  $\beta$ -galactosidase. These active site mutations were developed at the following residues: H296, E297, S360, F365, N412, E413, Y468, and E504, D163, V274, Q507, F281, K277. The subunit interface mutations were performed at the following residues to reduce electrostatic interactions: E6, R71, K13, D16, D319, R43, D77, T7, D477, K157, Y517, D53, R10, K77, N308, E523, H514, K576, and S579. In the future, MC-PRO by Schrodinger will be used to determine feasible mutations for laboratory testing. Through these calculations, mutations will be developed and studied for the interactions between *E. coli*  $\beta$ -glucuronidase and ligands in hopes of learning more about the enzyme mechanism and dimer stability.

## Computer Science

NONTRADITIONAL APPLICATIONS OF AUTOMATA THEORY. Bruce Chittenden, Department of Computer Science, Hampton University, Hampton, Virginia 23668. Automata theory is typically taught as a mathematical theory of computation with applications in compiler construction, specifically in the area of lexical analysis. Typically computer science students do not encounter automata theory outside of a course on programming languages or compiler construction, and are therefore left with the impression that automata theory is a fairly abstract mathematical theory which only applies in these areas. I would like to put forward the argument that we should expand how we teach automata theory to include several practical applications such as the specification and verification of protocols. Automata theory provides a rich set of abstractions and tools that every student should learn to apply to many situations that they will encounter in the development of complex software. I have found that automata theory is well suited for the specification and verification of many types of protocols. Any software situation where communications is occurring and state is maintained over a period of time lends itself to a software design and implementation based upon automata theory. The finite state machine is specified symbolically, that is the *inputs*, *outputs*, *states*, and *transitions* are defined in terms of symbols easily understood by humans but difficult in that form, to be used directly by a computer. An Analyzer Program can easily be created that processes the *description* of the Finite-State Machine and generates two tables of integers, *outputs* and *next state*, representing the machine behavior. These tables can be used directly by the computer. The software implementation becomes completely table driven. Using today's programming languages such as C, C++, or Java, this is easily implemented using a switch statement. The significant benefit of this approach is that it pushes the debugging of the protocol from coding and testing phase back into the design phase, therefore reducing the time to deliver working software.

OPEN QUESTIONS REGARDING UPPER BOUND ON MATRIX MULTIPLICATION OF  $O(n^w)$  FOR  $w < 2.374$ . S. V. Providence, Department of Computer Science, Hampton University. Matrix multiplication is a fast algorithm that can be distributed and has fast performance on modern computer systems. A lower

bound of  $W(n^2)$  for this algorithm holds even for sparse matrices. The best known lower bounds are  $c \times n^2$ , for constants  $c, b \geq 3$ . Strassen suggests matrix multiplication requires strictly more than quadratic time. Several categories for finding an upper bound to this algorithm persist: bilinear algorithms, approximate algorithms and Schönhage's theorem, ultra fast solutions to tensor products, and Coppersmith-Winograd algorithm. We examine these approaches with regard to numerical accuracy and stability while considering the current open questions and a possible choice for solution.

**A FLUID-BASED STOCHASTIC APPROACH FOR ANALYZING AND MODELING NETWORK TRAFFIC.** Yen-Hung Hu, Department of Computer Science, Hampton University, Hampton, VA 23668. In this paper, a fluid-based stochastic approach for modeling normal and malicious network activities is investigated and developed. To achieve the goal of this paper, several existing raw network traffic traces are analyzed first. And then a fluid-based stochastic network model for simulating normal and malicious network traffics according to characteristics of such raw traffic traces is investigated and developed. Since observing that the majority of the collected network traffic traces demonstrates self-similar behavior, this paper investigates this self-similar characteristic and adopts it as a key factor of identifying malicious activities. Several simulation results are included and the raw traffic traces shown in this paper are gathered by the Cooperative Association for Internet Data Analysis (CADIA).

## Education

**THE WAY FORWARD: UNDERSTANDING OUR ECOLOGICAL PLACE.** Emma D. Burnett<sup>1</sup>, Alana S. Burton<sup>2</sup> & Eugene G. Maurakis<sup>1</sup>, <sup>1</sup>Department of Biology, University of Richmond, Richmond VA 23173, <sup>2</sup>Department of Geology, College of William and Mary, Williamsburg VA 23185. This documentary film explores the modern relationship between Americans and the natural world by examining the perspectives of individuals with unique personal and professional connections to the environment. Interviews conducted with these individuals illuminate numerous facets of our relationship with the environment, from a farmer's reasons for investing in the long-term health of the environment, to a scientist's perspective on the role of skepticism in the pursuit of truth and the biggest challenges to educating the public. Some of the general questions addressed include the implications of our attitude towards the environment on our health and the health of future generations, challenges to the pursuit of general knowledge and understanding, including polarized viewpoints and special interest groups, and potential steps in overcoming these challenges. Through the examination of these topics by interview subjects from different backgrounds and occupations, the film presents various ways of thinking about the environmental problems that we face and highlights the importance of individual action and constructive dialogue.

**ENVIRONMENTAL ISSUES AND AMERICAN ATTITUDES.** Alana S. Burton<sup>1</sup>, Eugene G. Maurakis<sup>2</sup>, and David V. Grimes<sup>3</sup>, <sup>1</sup>College of William and Mary, <sup>2</sup>Dept. of Biology, University of Richmond, & <sup>3</sup>Old Dominion Appalachian Trail Club. In July

2012 the “Survey on Human- Environment Interaction” was launched using SurveyMonkey. The survey aimed to explore how humans view their relationship to the natural world as well as how demographics influence beliefs about environmental issues. In September, with 383 respondents, the survey was closed for analysis. Over 90% of respondents agreed that there is a direct link between human health and environmental health and that environmental degradation is disturbing; therefore, this consensus may serve as a useful backdrop to environmental education. Age, education level, and political affiliation were found to play an important role in how humans view environmental issues; therefore, these characteristics may be useful in the creation of educational programs.

CURRICULUM MATERIALS AS A COMPANION TO A LARGE MUSEUM EXHIBITION ON HUMAN HEALTH AND WELLNESS. David B. Hagan, Science Museum of Virginia, 2500 W. Broad Street, Richmond, VA 23220. The Science Museum of Virginia has a statewide mission for informal science education. It has spent 14 months in research, planning and design for an 8,000 square foot interactive exhibit gallery focused on the human body and mind. It also produced a five-minute education curriculum video on the human body, its systems, chemistry, and physiology. Research on the function and visitor learning was conducted on the interactive exhibits using formative evaluation. Student groups (elementary through high school) were observed engaging with a selected set from each of the three main categories of exhibits, (Body, Mind and Play). The results of these evaluation eyeles were used to implement modifications to the interactive exhibit units. In the Body section the exhibits included: an isometric bench press, a test of strength in pushing a fixed bar in a standing position; a test of balance on a 12-foot tightrope six inches above the floor; a test of reaction time in response to visual stimuli; a test of the visitor's ability to assume a yoga position and hold it for ten seconds; a test of the visitor's flexibility as measured by their ability to fit into a small space; and three exhibits focused on concepts related to nutrition, diet and food preparation in the Kitchen Theater. Visitor experiences in the Mind section include psychology-related engagements: a self-reported measure of happiness; two different short-term memory tests; a challenge of the visitor's capability to cooperate in a group to succeed at a timed challenge; a demonstration of eye-tracking sensors showing where the visitor's gaze was focused on an image; and a Sleep Theater experience that takes the visitor on a narrated journey through a typical night's sleep showing the sleep cycles and levels of sleep. The Play section of the exhibit includes: playing percussion instruments, attempting to match and hold a given note as a test of perfect pitch; and an interactive overview of pastimes, hobbies and sports. The exhibit has been tested and is in the process of installation.

### **Environmental Science**

PLANTS AS INDICATORS OF DISTURBANCE AT THE ABRAMS CREEK WETLANDS, A PROTECTED CALCAREOUS ECOSYSTEM IN WINCHESTER AND FREDERICK COUNTY, VIRGINIA. J. T. Walker, V. Thomas, W. S. Bousquet, D. F. Cooper, B. Ridgeway, S. Dieudonné, S. Kochman, J. Miller & G. Ayers. Environmental Studies Program, Shenandoah University, Winchester, Virginia 22601.

A 1998 study by Shenandoah University (SU) supplemented investigations by the Virginia Natural Heritage Program in documenting the ecological significance of the Abrams Creek Wetlands. In the spring and fall of 2012 SU researchers resurveyed four communities from the 1998 study. Using the relevé (Braun-Blanquet) method, community plots were described by their vegetation and physical characteristics. For each community, researchers compared the changes in relative cover by species from 1998 to 2012. Indicator plant species were selected by three criteria: large ( $\geq 3$  s.d.) change in relative cover, rarity in Virginia, and low coefficient of conservatism. Factors potentially responsible for changes in vegetation were then identified. While the analysis is preliminary, it provides opportunities to better understand wetland ecology, improve wetland management, and protect local and regional biological diversity.

STORMWATER MITIGATION CHOICES BY THE GENERAL PUBLIC. Shane H. Abinette<sup>1</sup> & Eugene G. Maurakis<sup>2 & 3</sup>, <sup>1</sup>Biology Dept., Virginia Commonwealth University, <sup>2</sup>Science Museum of Virginia, & <sup>3</sup>Biology Dept., University of Richmond. Objectives were to educate and survey visitors in the Rainkeepers exhibition at the Science Museum of Virginia regarding proper stormwater mitigation practices. Survey data were gathered from visitors' voting responses in interactive panels that asked 11 questions regarding the user's habits on littering, car washing, oil recycling and other topics from December 2012 –April 2013. Results showed a high visitor willingness to use pervious concrete and rain barrels but a lack of actual use of these materials. High levels of participation in litter and pet waste pick up, and reduced use of lawn pesticide were also found. A high willingness was also detected for recycling motor oil and converting lawns to native vegetation. For all questions, a majority of respondents claimed they already did or were willing to practice good stormwater management techniques. Funded by a National Fish and Wildlife Foundation grant.

LAND-USE PROBLEMS FOR LOCAL AIR QUALITY AND BIOLOGICAL CONSERVATION, A SIMPLE SOLUTION PROPOSED. Richard S. Groover, Department of Environmental Science and Policy, George Mason University, Fairfax VA 22030-4444. Local air quality and biological conservation is connected to land-use decisions and resulting changes. The citizens and the governmental officials often may not understand the importance of air quality or biodiversity conservation, and ecosystem connectivity with respect to their land-use decisions, but they need to become aware of such. Individuals or developers who wish to maximize their use of land and resources often have less concern for conservation. This presentation explores the current situation local habitat destruction and how these losses impact air quality, local biota in Virginia, how local governments are structured, local government decision-making processes, and what conservation supporting tools exist for them to use. Two win/win strategies for achieving better success with local air quality and biological conservation can improve local land-use decisions. One of the strategies is the development of an innovative proffer and mitigation approach to increase trees and improve carbon sequestration on land proposed for development. It includes an in-perpetuity protection of set-a-side forest areas. The second strategy involves an in-perpetuity protection of wildlife travel corridors, within the associated land being



rezoned. Both strategies would be utilized during the zoning change phase for property to be developed.

#### Posters

A COMPARATIVE ANALYSIS OF THE HEALTH OF THE NI RIVER AND MASSAPONAX CREEK IN SPOTSYLVANIA COUNTY. Dr. M. L. Bass, E. E. Stewart, A. T. Elliott & H. E. Richters, University of Mary Washington. The main focus of this research project was to determine the health of both the Ni River and Massaponax Creek in Spotsylvania County, VA. The two streams provide a good demonstration of the effects of urban development. There are four sample sites located on Massaponax Creek and three sites on the Ni River. At each site, macrobenthic samples were taken as bio-indicators of pollution. These samples were corroborated by water chemistry analyses, including conductivity, dissolved oxygen levels, fecal coliform, alkalinity, calcium hardness, phosphate and nitrate concentrations and total dissolved solids. All samples were taken within each season. An examination of the macrobenthic results indicate that throughout the summer, the %EPT of the Ni River was approximately 90% at most sites. The low was of 82.8% at the McEwan Farm site. At Massaponax Creek, the summer's %EPT ranged from 97.3% at the Wetland to a low of 62.7% at Route 208. These ranges are caused by variations in water chemistry. During autumn and winter, the %EPT's steadily fell, as the organisms completed their aquatic life cycles. It can also be noted that one particular site on Massaponax was afflicted by a high CFU count, as well as high spikes in alkalinity, phosphate and hardness in the winter. It has been determined that the Ni River is healthier than Massaponax Creek. This is represented by the higher EPT percentages and diversity of macrobenthic individuals within the Ni River. However, Massaponax Creek has maintained good health despite increased development within its watershed.

### Medical Sciences

THIOREDOXIN-INTERACTING PROTEIN MEDIATES HCYS-INDUCED NLRP3 INFLAMMASOME ACTIVATION IN MOUSE PODOCYTES. Justine M. Abais, Krishna Boini, Min Xia, & Pin-Lan Li, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Our recent studies have demonstrated that NADPH oxidase-derived reactive oxygen species (ROS) activates NLRP3 inflammasomes causing homocysteine (Hcys)-induced podocyte and glomerular injury, however the precise mechanism regarding how ROS activates the inflammasome is still unknown. The current study explored whether thioredoxin-interacting protein (TXNIP) mediates Hcys-induced NLRP3 inflammasome activation in podocytes. TXNIP, the regulatory inhibitor of the antioxidant thioredoxin (TRX), is thought to dissociate from TRX in response to elevated levels of oxidative stress to bind to inflammasome protein NLRP3 and activate the inflammasome complex. Genetic or pharmacologic inhibition of TXNIP by small interfering RNA or verapamil prevented Hcys-induced NLRP3 inflammasome formation and activation both *in vitro* and *in vivo* by reducing colocalization of NLRP3 with ASC or caspase-1, blocking Hcys-induced coimmunoprecipitation of TXNIP with NLRP3, and diminishing

caspase-1 activity and IL-1 $\beta$  production. In addition, TXNIP inhibition protected podocytes from Hcys-induced injury as demonstrated by normal expression levels of podocyte markers podocin and desmin, and preservation of glomerular function by reduced proteinuria and albuminuria *in vivo*. These results concluded that TXNIP binding to inflammasome protein NLRP3 is a key signaling mechanism for Hcys-induced NLRP3 inflammasome formation and activation, and subsequent glomerular injury (supported by NIH grants DK54927 and 1F31AG043289).

THE BALANCE OF T-LYMPHOCYTE SPECIFYING TRANSCRIPTION FACTORS AND THE CYTOKINE EXPRESSION IN OBESE PATIENTS WITH NAFLD. M. Keaton<sup>2</sup>, R. Mehta<sup>1,2</sup>, A. Neupane<sup>2</sup>, & A. Baranova<sup>1,2</sup>. <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova, Falls Church VA 22042 & <sup>2</sup>School of Systems Biology, George Mason Univ., Fairfax VA 22030. The accumulation of immune cells within visceral adipose tissue (VAT) in subjects with NAFLD has been poorly understood. The immune response in obesity is reported to involve the shift in ratio of subsets of T cells (T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>17) within VAT through the transcription factors, TBX21, GATA3 and ROR $\gamma$ , respectively. The aim was to assess the ratio of T-cell lineage transcription factors and T-cell associated cytokines in obese patients with NAFLD. Forty-five biopsy-proven NAFLD patients' VAT samples were collected. RNA was extracted, and then converted, to cDNA. RT-PCR profiling was performed using validated primers for T cell lineage specifying markers (CD3E - CD4+ cell marker; TBX21 - Th1; GATA3 - Th2; FOXP3 - Treg). ACTB was used for normalization. Group comparisons for ratios like TBX21/CD3E and TBX21/GATA3 were carried out amongst all possible cohorts. There were consistently higher ratios of GATA3/CD3E compared to TBX21/CD3E and FOXP3/CD3E within each histological cohort related to grade of steatosis, histologic NASH, and stage of fibrosis. Group comparison of cytokines showed TGF-B1 (FC=0.13, p=0.04) and IL-1B (FC=0.79, p=0.03) were down-regulated in patients with NASH. IL-1B (FC=0.25, p=0.007) was down-regulated in patients with advanced hepatic fibrosis. The immune imbalance that is seen in obese NAFLD patients does not seem to depend on the expression of transcription factors involved in T cell lineage determination in the adipose tissue.

PRECLINICAL ASSESSMENT AND SPECTROSCOPIC CHARACTERIZATION OF THE ABUSED SYNTHETIC CANNABINOID, CP47,497. K. L. Samano<sup>1</sup>, L. E. Wise<sup>1</sup>, J. L. Poklis<sup>1</sup>, A. Poklis<sup>1,2,3</sup> & A. H. Lichtman<sup>1</sup>, <sup>1</sup>Dept. of Pharmacology & Toxicology, <sup>2</sup>Dept. of Forensic Science & <sup>3</sup>Dept. of Pathology, Virginia Commonwealth University, Richmond, VA, 23298. The objective of the present study was to characterize the pharmacological consequences of the abused synthetic cannabinoid compound, CP47,497. Cannabimimetic effects were assessed using the well-established tetrad model that is sensitive to the chief psychoactive compound present in marijuana, THC, and consists of four outcome measures: catalepsy, antinociception (tail flick latency), hypothermia, and locomotor activity. CP47,497 was significantly more potent than THC in each measure. The CB<sub>1</sub> receptor antagonist, rimonabant, reversed all pharmacological effects of CP47,497. Consistent with these findings, CB<sub>1</sub> (-/-) mice were resistant to the cannabimimetic effects of CP47,497. Immediately following behavioral testing, mice were humanely euthanized and blood and tissue were harvested for CP47,497 quantification. Samples are currently being

analyzed on an Applied Biosystems Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS) interface utilizing electrospray ionization and selective ion monitoring, using acetonitrile liquid-liquid extraction. Additionally, ongoing research is examining tolerance and dependence liability, as well as CB<sub>1</sub> receptor adaptations following repeated administration of CP47,497. In conclusion, the results of the present study indicate that acute administration of CP47,497 elicits markedly more potent CB<sub>1</sub> receptor mediated effects (i.e. 5-9 fold) than THC.

PRESERVATION OF T CELL FUNCTION AS A NECESSARY COMPONENT IN CHEMOTHERAPEUTIC STRATEGIES IN TREATMENT OF MELANOMA TUMORS. Se W. Jeong & Timothy N. J. Bullock, Dept. of Pathology, University of Virginia, Charlottesville, VA 22908. Metastatic melanoma has a poor prognosis as patients are only expected to live six to twelve months after diagnosis. PLX4720 is a specific inhibitor that targets a B-Raf gene mutation that is characterized by a V to E substitution at position 600. This has been highly anticipated as more than half of the patients present this mutation. While PLX4720 has had dramatic short-term success, the tumors escaped the B-Raf inhibition through other proliferative pathways. Therefore, six additional inhibitors were identified to have synergistic cytotoxicity with PLX4720 against melanoma tumors. This project probed how these combinations of chemotherapies, that have proven synthetic lethality against melanoma, impacted upon T cell function and cytokine expression. Ultimately, the goal was to define the least detrimental combinations of drugs to T cells for combined chemotherapy/immunotherapy approaches. Although most inhibitor combinations were not deleterious, only one combination, PLX4720 and Masitinib, a platelet-derived growth factor receptor inhibitor, was detrimental to T cell effector function. Therefore, our data indicates that it is possible to select multi-model therapy using combinations of chemotherapies and immunotherapy.

hOCTs: NOVEL TARGET FOR ANTIDEPRESSANT THERAPY. Kavita Iyer<sup>1</sup>, Xiaolei Pan<sup>2</sup>, Osama Alwassil<sup>1</sup>, Douglas Sweet<sup>2</sup>, & Malgorzata Dukat<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry & <sup>2</sup>Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, Richmond VA 23298. Drugs that target norepinephrine and serotonin transporters (NET and SERT, respectively) are commonly utilized to treat depression, however they have certain drawbacks associated with them. There is an acknowledged need to identify novel antidepressants. Human organic cation transporters (hOCTs) are proteins that modify the transport of endogenous neurotransmitters such as serotonin and norepinephrine. Recently, antidepressants have been shown to interact with hOCTs and may be a potential target for drug development. Our laboratory has synthesized a series of quinazoline analogs that are constrained analogs of *m*-chlorophenylguanidine (MD-354), a 5-HT<sub>2</sub> receptor partial agonist. Two quinazoline analogs (KEO-099 and KEO-147) were found to be 5-HT<sub>2</sub> receptor antagonists and tested positive in the mouse tail- suspension test. However, the two analogs differed in their ability to bind to 5-HT<sub>2</sub> receptors as well as NET and SERT. It was concluded that neither NET nor SERT are able to adequately explain the antidepressant effects of the quinazoline analogs. This led us to examine these analogs at hOCTs to determine if their action at hOCTs could contribute to their

antidepressant effects. Results from the preliminary tests indicate that quinazolines inhibit the transport of 1-methyl-4-phenylpyridine (MPP<sup>+</sup>), a model substrate for hOCTs. Moreover, we have established a rudimentary structure-activity relationship (SAR), which identifies substituents seemingly important for both binding as well as selectivity for hOCT subtypes.

**NLRPS IN VISCERAL ADIPOSE TISSUE OF MORBIDLY OBESE PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD).** Arpan Neupane<sup>2</sup>, Rohini Mehta<sup>1,2</sup>, Ancha Baranova<sup>1,2</sup>, Arian Afendy<sup>1</sup>, Zachary Goodman<sup>1</sup>, & Zobair Younossi<sup>1</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA & <sup>2</sup>School of Systems Biology, George Mason University, Fairfax, VA. Inflammasome-associated Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain containing (NLRP) family proteins serve as intracellular sensors of PAMPs (toxins, whole pathogens, etc.) and DAMPs (dsDNA, cellular debris, etc.). Inflammasome mediated activation of caspase-1 leads to cleavage of pro-IL1B and pro-IL18 and in turn induce expression of a number of cytokine genes triggering a cascade of inflammatory and immune responses. Obesity is a state of low-grade chronic inflammation and inflammation has been shown to play an important role in obesity-associated non-alcoholic fatty liver disease (NAFLD). NAFLD is strongly associated with visceral obesity. In this study we investigated the expression of 14 NLRPs genes in visceral adipose tissue of morbidly obese subjects (N=46) with NAFLD. Expression of 11 NLRPs members was not detected (Ct ≥ 35). Interestingly, NLRP1 and NLRP3 (the most well studied NLRPs) were found to be downregulated in morbidly obese subjects with severe NAFLD. This study for the first time determines the expression of NLRPs in visceral adipose tissue of morbidly obese subjects with NAFLD. The results show downregulation of inflammasome sensors with increasing severity of obesity-associated NAFLD. Further studies are needed to dissect the mechanism of NLRP downregulation.

**COMBINED ADMINISTRATION OF THE SELECTIVE FAAH INHIBITOR PF-3845 AND COX INHIBITOR DICLOFENAC: INVESTIGATION IN A MURINE MODEL OF NEUROPATHIC PAIN.** T. W. Grim<sup>1</sup>, S. O'Neal<sup>1</sup>, S. J. Kinsey<sup>2</sup>, J. M. Pearson<sup>3</sup> & A. H. Lichtman<sup>1</sup>, <sup>1</sup>Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, <sup>2</sup>Department of Psychology, West Virginia University, Morgantown, VA, & <sup>3</sup>Ironwood Pharmaceuticals, Inc. Cambridge, MA. Fatty acid amide hydrolase (FAAH), the primary degradative enzyme for the endogenous cannabinoid anandamide, offers a novel target to attenuate neuropathic pain, a condition refractory to existing analgesics. In the present study, we employed the chronic constriction injury model of neuropathic pain (CCI) to assess whether combined administration of the FAAH inhibitor, PF-3845, and the nonselective cyclooxygenase inhibitor (COX) inhibitor, diclofenac, would produce augmented antinociceptive effects. Dose response studies were conducted for each compound and subthreshold doses were given in combination to test for augmented antinociceptive effects in the CCI model. The co-administration of 5 mg/kg PF-3845 and 30 mg/kg diclofenac produced significant antinociception compared with either drug given alone. Metabolomic analysis found that PF-3845 elevated brain anandamide levels and

diclofenac reduced prostaglandin brain levels, regardless of whether the compounds were given separately or in combination.

PHOSPHORYLATION OF RAPTOR FOLLOWING PEMETREXED ACTIVATION OF AMPK OVERCOMES TSC2 DOWN REGULATION AND mTORC1 ACTIVITY. Stuti Agarwal, Catherine Bell, & Richard Moran, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Pemetrexed is a highly unusual antifolate in that it has significant therapeutic utility against non-small cell lung cancers (NSCLC) and mesothelioma. We have shown that pemetrexed has a second mechanism, in addition to inhibition of thymidylate synthase, which leads to robust activation of AMP-dependent kinase (AMPK), and have suggested that this effect is causal of the activity of this drug against lung cancers. The most frequently mutated gene in NSCLC is p53 and, yet, the effects of pemetrexed are retained in p53-null carcinoma cells. Like K-Ras mutation and EGFR alterations, p53 loss causes hyperactivity of mTORC1; this is due primarily to loss of TSC2 function. The effects of pemetrexed also result in a functional loss of transcriptional activation by p53, with resultant loss of TSC2 function and concomitant expansion of RhebGTP. However, the robust stimulation of AMPK phosphorylation of Raptor in pemetrexed-treated cells overwhelms these effects and causes suppression of mTORC1.

IL28B GENOTYPE ASSOCIATIONS WITH METABOLIC SYNDROME. Leah Byars<sup>2</sup>, Ancha Baranova<sup>1,2</sup>, James M. Estep<sup>1,3</sup> & Zobair Younossi<sup>1,2,3</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA & <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital. Hepatitis C Virus (HCV) is a viral infectious disease that affects an estimated 130-170 million people. In a number of recent studies, the *IL28B* gene has been shown to play an important role in the outcome of HCV treatment. Metabolic syndrome (MetS) is a group of medical disorders usually associated with obesity. Recent, but not conclusive, evidence suggests that *IL28B* genotypes may be associated with metabolic confounders of HCV. In HCV patients, there is a significant correlation between the presence of CC genotype and several metabolic factors. In non-HCV patients with NAFLD, no such correlation was found. The metabolic profiles of the CC and Non-CC groups of NAFLD patients were also not different from each other. The beneficial effects of CC genotype on metabolic profiles may only be conferred after incidental infection with HCV, or after the treatment with antiviral therapy. *IL28B* genotyping in larger groups of non-HCV patients is warranted to determine the true effect of *IL28B* genotypes on metabolic outcomes.

ACTION OF DECONSTRUCTED MDPV ANALOGS AT hDAT. F.T. Sakloth<sup>1</sup>, R. Kolanos<sup>1</sup>, E. Solis<sup>2</sup>, L. De Felice<sup>2</sup> & R. A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298 & <sup>2</sup>Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond VA 23051. The number of cases reported on the abuse of psychoactive “bath salts” has intensified in the past few years. These “synthetic cathinones” are  $\beta$ -keto analogs of amphetamine and were scheduled as of October 21, 2011. Methylenedioxypyrovalerone

(MDPV), a major constituent of bath salts, is the first synthetic cathinone known to act as a dopamine re-uptake inhibitor, i.e. to show a hyperpolarizing current. The purpose of this project was to understand which structural features contribute to its hyperpolarizing action, and determine what is it about MDPV that converts it from a depolarizing to a hyperpolarizing agent? MDPV was deconstructed into seven analogs, making only one structural change at a time. All the analogs, as with MDPV, showed a hyperpolarizing current in electrophysiological studies using frog oocytes transfected with hDAT. The  $IC_{50}$  values were measured and they ranged over a 200-fold span. Amongst the structural features that contribute to affinity, the tertiary amine and/or the extended side chain are optimal for affinity, but the extended side chain seems critical for affinity. However, the carbonyl oxygen atom and the methylenedioxy ring are not major contributors to affinity and not required for the hyperpolarizing action. In addition to understanding the structural contribution of MDPV to its hyperpolarizing action we are the first to report on the mechanism of newly confiscated MDPV analogs. [Supported by NIH grant DA 5R01DA033930.]

ENDOCANNABINOID METABOLIC INHIBITORS FOR THE TREATMENT OF AFFECTIVE ASPECTS OF MORPHINE WITHDRAWAL. T. F. Gamage & A. H. Lichtman, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Inhibition of the endocannabinoid catabolic enzymes fatty acid amide hydrolase (FAAH) with PF3845 or monoacylglycerol lipase (MAGL) with JZL184 reduces morphine withdrawal signs. It is uncertain if these anti-withdrawal effects extend to aspects related to the affective components of withdrawal. In the present study, we tested whether FAAH or MAGL inhibition would block the acquisition of conditioned place avoidance (CPA) to morphine withdrawal. While morphine (30 mg/kg) blocked acquisition of CPA and reduced jumping, neither JZL184 (4 or 40 mg/kg), PF3845 (1, 3, or 10 mg/kg), nor clonidine (0.03-1 mg/kg) affected acquisition of naloxone-precipitated morphine withdrawal-induced CPA. Additionally, delta-9-tetrahydrocannabinol (1, 3, or 10 mg/kg) did not affect withdrawal CPA. However, both JZL184 and THC significantly reduced the percentage of mice that exhibited jumping behavior. These data suggest that while inhibition of endocannabinoid metabolism reduces overt behavioral withdrawal signs, it is ineffective at reducing the aversive aspects of withdrawal. Thus, these drugs only reduce a subset of aspects related to opioid withdrawal. Future studies will examine the effects of combination treatment of dual FAAH/MAGL inhibition on acquisition of withdrawal CPA as well as other affective-related withdrawal behaviors.

#### Posters

ADIPONECTIN PREDICTOR OF LONG-TERM SUSCEPTIBILITY TO OBESITY. E. Jenkie<sup>1</sup>, J. Bahamonde<sup>2</sup>, B. Brenseke<sup>2</sup>, M. Friedman<sup>1</sup>, L. Mitchell<sup>1</sup> & R. Prater<sup>1,2</sup>, <sup>1</sup>EVCOM; <sup>2</sup>VMRCVM. In 2009-2010, 35% of adult Americans were obese and at high risk for metabolic disease due to sedentary behavior and chronic overindulgence of fat and carbohydrates. Adiponectin is a protein hormone secreted by adipose tissue and is inversely correlated with body fat percentage in adults. It aids in the suppression of metabolic derangements that may result in obesity, type 2 diabetes, atherosclerosis and metabolic syndrome. Our laboratory proposed that maternal malnutrition, represented

as high fat diet (HFD), high sugar diet (HSD) or ethanol consumption (EtOH) throughout pregnancy, would elevate long-term risk of obesity in mouse offspring and that adiponectin in offspring from dams exposed to these diets could be measured in order to predict long-term susceptibility to obesity. Liver adiponectin was measured in control, HFD, HSD and EtOH groups at postnatal day 1, 21 and 42 in C57BL/6 mice using a mouse adiponectin ELISA. Serum insulin levels were measured, and histopathology reports and x-ray microtomography images were obtained for disease detection. Adiponectin decreased with age in all groups. The treatment groups had lower adiponectin levels (HFD group was lowest), compared to controls. Additionally, HFD offspring were found to have higher insulin levels than controls, and their femurs displayed fat accumulation and lesions resembling osteoporosis. Findings suggest that prenatal malnutrition can lead to diminished adiponectin levels and that measuring adiponectin may help to predict future risk of obesity and related disorders. This biomarker may then be manipulated in novel ways (e.g., nutritional interventions) in order to improve long-term health.

THE EFFECTS OF GLIAL CELL MODULATORS ON METHAMPHETAMINE (METH) INTRAVENOUS SELF-ADMINISTRATION AND FOOD-MAINTAINED BEHAVIOR IN RATS. S. E. Snider<sup>1</sup>, E. S. Hendrick<sup>1</sup> & P. M. Beardsley<sup>1,2,3</sup>, <sup>1</sup>Department of Pharmacology and Toxicology, <sup>2</sup>Institute for Drug and Alcohol Studies, <sup>3</sup>Center for Biomarker Research and Personalized Medicine, School of Pharmacy, Virginia Commonwealth University, Richmond Virginia. Methamphetamine (METH) administration activates glial cells in the CNS and increases pro-inflammatory cytokine production and release. Glial cell activation and pro-inflammation have been linked to drug abuse-related behavior. Ibudilast (AV411; 3-isobutryl-2-isopropylpyrazolo-[1,5-a]pyridine), which inhibits phosphodiesterase (PDE) and glial-mediated pro-inflammatory activity, AV1013, an amino analog of ibudilast with similar glial but limited PDE activity, and minocycline, a tetracycline derivative with similar glial attenuating capabilities, all attenuate METH (0.03 mg/kg/inf) self-administration. The effects of ibudilast (10 mg/kg) and minocycline (30 & 60 mg/kg) on food-maintained responding were also examined using a behavioral economic approach. Demand curves for both METH (0.03 mg/kg/inf) and food-maintained behavior were obtained by increasing the FR value daily using the following progression: 1, 3, 6, 9, 13, 19, 26, 35, 47, 62, 82, 108. After matching the essential values ( $\alpha$  levels) of METH and food pellets, twice daily ibudilast or once daily minocycline, was administered for three days under FR 1 conditions. Ibudilast and minocycline both significantly ( $p < 0.05$ ) reduced both METH- and food-maintained responding. These results indicate that ibudilast and minocycline have complex effects potentially able to attenuate behavior maintained by multiple events.

BLOCKADE OF THE NMDA RECEPTOR ION CHANNEL ENHANCES THE DISCRIMINATIVE STIMULUS EFFECTS OF NITROUS OXIDE. Kellianne J. Richardson & Keith L. Shelton, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23284-2006. The abuse-related CNS effects of nitrous oxide ( $N_2O$ ) are poorly understood. *In vitro* data suggests that  $N_2O$  alters the function of NMDA and GABA<sub>A</sub> receptors, amongst others. Our goal was to

assess the neurotransmitter systems responsible for producing the intoxicating effects of  $N_2O$  using drug discrimination. Twenty-four mice were trained to discriminate 10 min of exposure to 60%  $N_2O$ /40%  $O_2$  versus 100%  $O_2$  in daily 5 min operant sessions. Mice acquired the discrimination in an average of 38 days.  $N_2O$  produced concentration-dependent substitution for the training concentration. Full substitution required 7 min of 60%  $N_2O$  exposure but the offset of stimulus effects  $N_2O$  following the cessation of exposure was more rapid. In substitution studies the NMDA channel blocker MK-801 produced up to 49%  $N_2O$  lever-selection at 0.56 mg/kg. While MK-801 failed to produce more than partial substitution, an intermediate dose of 0.17 mg/kg MK-801 significantly shifted the  $N_2O$  concentration-effect curve to the left. The competitive NMDA antagonist CGS 19755 and the positive GABA<sub>A</sub> modulator midazolam failed to substitute for  $N_2O$ . The stimulus effects of nitrous oxide also partially overlap with volatile solvents and anesthetics. Taken together these data suggest the subjective stimulus properties of  $N_2O$  may be partially mediated by NMDA antagonism. Other receptor systems not yet examined are probably also involved. Supported by NIDA grant RO1-DA020553.

THE EFFECTS OF WESTERN-STYLE DIET ON ATTENTIONAL SET SHIFTING PERFORMANCE. S. Marwitz, L. Woodie & S. Blythe, Dept. of Biol., Washington & Lee University, Lexington, VA 24450. The rate of obesity in the human population has risen to alarming levels in recent years. Obesity has been linked with a number of maladies such as insulin resistance, leptin resistance and diabetes. However, there is now growing evidence that obesity may even be linked to hippocampal damage. We conducted a pilot study to find if an attentional set shifting task could detect damage done to the hippocampus from a high fat diet. We used two groups of rats, one maintained on a high fat diet (n=19) and the other maintained on a control diet (n=19). The diets continued for 11 weeks when we performed the attentional set shifting task. Before the attention task the rats were maintained on limited versions of their respective diet so that they reached 85% of their body weight. In the task the rats were presented with two identical glass cups filled with a different material and scent. Rats were required to remember which scent or material was baited, with tasks increasing in difficulty. We found that diet did not have a significant effect on performance in the task ( $p=0.177$ ,  $p=0.751$ ). However, there was significant decline in performance as the tasks progressed and became more difficult ( $p=0.052$ ,  $p=0.033$ ). With this data and further research into other attention tasks, we hope to establish an appropriate test to shed light on the effects of a high fat diet on the brain. (Supported by: Howard Hughes Medical Institute Fund, Levy Foundation Grant, The Virginia Academy of Sciences and Washington & Lee University.)

STATISTICAL ANALYSIS OF ENVIRONMENTAL RISK FACTORS OF BREAST CANCER. Amirhossein Shamsaddini<sup>1,2</sup>, Ancha Baranova<sup>1,2</sup>, Aybike Birerdinc<sup>1,2</sup>, <sup>1</sup>School of Systems Biology, College of Science, George Mason University & <sup>2</sup>Betty and Guy Beatty Obesity and Liver Program, Inova Health System, Falls Church, VA. Breast cancer is the most frequently diagnosed cancer among women, accounting for about 30% of all new cancer cases each year. In the early 1990s, it was suggested that exposure to some environmental chemicals such as organochlorine compounds (OC) may play a causal role in the etiology of breast cancer through estrogen-related



pathways. We used 1999–2009 National Health and Nutrition Examination Survey data to examine associations between serum concentrations of OC pesticides and breast cancers. Statistical analysis (*Logistic Regression*) was performed with SPSS v.20 on these databases after carefully screening for any inconsistency. Although further study is necessary to confirm these findings, these statistical results suggest that OC pesticide exposures may have a significant effect on breast cancer risk.

HUMAN LEUKOCYTE ANTIGEN (HLA) CLASS I COMPLEX: CLINICAL SIGNIFICANCE AS AN INDEPENDENT PREDICTOR OF NON-ALCOHOLIC FATTY LIVER DISEASE. Ali Moosvi<sup>1,3</sup>, Azza Karrar<sup>2,3</sup>, Zachary Goodman<sup>2</sup>, Ancha Baranova<sup>1,2,3</sup> & Zobair Younossi<sup>1,2</sup>, <sup>1</sup> Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & <sup>3</sup>School of Systems Biology, College of Science, George Mason University, Fairfax, VA. The overall aim of our study is to assess the association between the Human Leukocyte Antigen (HLA) Class I Antigens polymorphism and hepatic steatosis, which is an independent predictor of cardiovascular disease. Patients with biopsy-proven Non-Alcoholic Fatty Liver Disease (NAFLD) (n =199) were genotyped with the PCR Sequence Specific Oligonucleotides (PCR-SSO) for the HLA-A,-B, and -C. NAFLD patients were grouped to steatohepatitis (NASH) and Non-NASH NAFLD. Univariate and multivariate analyses were performed to determine significant correlations. HLA – A11, -B51 were found to be strongly associated to high-grade hepatic steatosis. In multivariate analysis approach, our data demonstrated an independent association between HLA-A11 and lower risk of high-grade hepatic steatosis, in addition to an independent association between HLA-A31, -B64, -B57 and histologic NASH. Notable differences were seen within the HLA-C loci, where Cw7 correlated to a protective effect against Advanced Fibrosis, while Cw8 had a predisposing effect. Identified risk haplotypes will aid in management of NAFLD by stratifying patients into groups with increased risk in developing not only NASH or Advanced Fibrosis, but also cardiovascular diseases.

MATRICELLULAR PROTEINS AND THEIR SPLICE VARIANTS IN OBESE NAFLD PATIENTS. Z. Asif<sup>1,3</sup>, R. Mehta<sup>1,2</sup> & A. Baranova<sup>1,2</sup>, <sup>1</sup>Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & <sup>3</sup>Biology Department, College of Science, George Mason University, Fairfax, VA. Matricellular proteins (MPs) are extracellular proteins that modulate cell-matrix interactions and cell function. MPs are doing this by remodeling the extracellular matrix. Obesity is a chronic disease that has reached epidemic proportions worldwide. Obesity is accompanied by excessive storage of fat in adipose tissue and ectopic sites like liver. Obesity is associated with many chronic diseases, notably nonalcoholic fatty liver disease (NAFLD). During obesity, the adipose tissue undergoes extensive remodeling (hyperplasia and hypertrophy) to accommodate excessive fat deposition. This extracellular matrix remodeling is, in turn, associated with altered expression of MPs. Some of the MPs have alternate isoforms whose expression and roles have not been examined in the context of obesity and associated NAFLD. These isoforms may be released into circulation and may affect distant sites such as liver. The aim was to

conduct a literature survey and shortlist MPs implicated in obesity and fatty acid metabolism. A literature survey was done on MPs using PUBMED and Google Scholar. MPs of interest were shortlisted and primers were designed for the different variants. Among the MPs, primers were designed for osteopontin (OPN), angiopoietin-like 4 (ANGPTL4), and autotaxin (ATX). Their expression in obese patients with NAFLD was detected. Future work: To design primers specific to each mRNA isoform and to detect their expression in obese patients with NAFLD.

THE RELATIONSHIP BETWEEN DEPRESSION AND THE FATTY LIVER INDEX. Nayeem Hossain<sup>1,2</sup>, Michael Estep<sup>1,2,3</sup>, & Ancha Baranova<sup>1,2</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA, & <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA. The fatty liver index (FLI) uses various chemical and physical measurements. This study observed the effect of depression's chemical environment on these values, focusing on triglyceride level and body mass index (BMI). Mann-Whitney tests compared triglyceride levels and BMI of those with depression to those not depressed in the overall population; subpopulations diagnosed with NASH were compared for further analysis. Triglyceride levels of depressed individuals were significantly higher than those not depressed in the overall population ( $P < 0.0232$ ). The triglyceride value of individuals with both NASH and depression was not significantly higher ( $P < 0.1738$ ) than those with only NASH. The average BMI was not significantly higher in depressed individuals than those not depressed in both overall population ( $P < 0.3371$ ) and NASH subpopulation ( $P < 0.4122$ ). Elevated triglyceride levels cause the FLI of depressed individuals without NASH to be higher than those of individuals without depression or NASH. Greater waist circumference, attributed to elevated triglyceride levels, further affects FLI values.

THE GENE EXPRESSION PATTERNS IN THE ADIPOSE TISSUE OF METABOLIC SYNDROME PATIENTS IN THE CONTEXT OF GENDER. Ashley Greer<sup>1,2</sup>, Brianda Beverley<sup>1,2,3</sup>, Micheal Estep<sup>1,2,3</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, <sup>2</sup>CSCMD, GMU, Fairfax, VA, & <sup>3</sup>NVCC, Annandale, VA. Adipose tissue metabolism differs between men and women. Study of these differences may shed light into pathologies such as metabolic syndromes that affect men and women differently. The aim of this study is to examine mRNA and miRNA expression from visceral adipose tissue and circulating orexigenic hormones with respect to gender and obesity associated disease. Visceral adipose tissue (VAT) was collected from biopsy-proven NAFLD patients who were undergoing bariatric surgery (N=24, Male=6, Female=18). MiRNA expression data from the VAT of NAFLD patients were obtained. Differences in circulating concentration or expression was considered significant between men and women if the absolute value of the change was  $>1.5$  and a P-value of  $<0.05$  was obtained. Regarding the cohort in which miRNA expression was measured, of the males in this group, 33% of patients have a diagnosis of diabetes, and 50% have a diagnosis of non-alcoholic steatohepatitis (NASH). Many of the differentially expressed miRNA correlate significantly with clinical and laboratory data in women. The variation in the expression patterns between men and women support that there is a gender-dependent difference in morbid obesity.

Further analysis on the expressions and functions of these molecules is necessary. Further study of this gene could express a difference in growth regulation in men and women. Better understanding of the functions for these molecules may help to develop gender-specific screenings or treatments for morbidly obese men and women.

PARADOXICAL EFFECTS OF MORPHINE WITHDRAWAL IN MOUSE MODELS OF ANXIETY-LIKE BEHAVIOR. J. P. Crowley III, T. F. Gamage, & A. H. Lichtman, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Opioid dependence presents a serious threat to society and affects approximately 2.4 million individuals in the United States. Cessation of drug taking leads to a withdrawal syndrome consisting of somatic signs, flu-like symptoms, and anxiety, which contribute to relapse. Typical models of anxiety have a lack of face validity in terms of expression of anxiety-like behavior during opioid withdrawal. It's been shown that mice going through morphine withdrawal display an increased open-arm time in the elevated plus maze, a behavior typically interpreted as anxiolytic. We assessed morphine withdrawal in two assays typically used to assess anxiety, the light/dark box (LD box), a conflict task similar to the EPM, and novelty-induced hypophagia (NIH), which measures consumption of palatable food in the home cage and a novel environment. In this assay a decrease in consumption and/or increase in latency is interpreted as anxiogenic behavior. Male ICR mice were implanted with either 75 mg morphine or placebo pellets and tested 48 h later receiving either naloxone (0.03-0.1 mg/kg) to precipitate withdrawal or saline. Morphine withdrawal increased the time spent in the light side of the LD box and reduced the latency to consume food in the NIH test – effects typically interpreted as anxiolytic. The increased time in the light side might reflect a shift in defensive behaviors toward escaping. Additionally, naloxone may be blocking morphine's suppressive effects on food consumption thus manifesting as a decrease in latency to consume. Further studies will test the effects of withdrawal in assays with greater face validity for escape behavior.

LIPID, GLUCOSE AND LIVER ENZYMES PROFILING OF OBESE NAFLD PATIENTS. Sweta Sedhai<sup>1,2</sup>, Katherine Doyle<sup>1,2</sup>, Amir Shamsad<sup>1,2</sup>, Aybike Bircerdinc<sup>1,2</sup> & Ancha Baranova<sup>1,2</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA & <sup>2</sup>School of Systems Biology, College of Science, George Mason University, Fairfax, VA. Insulin resistance is a major and very common issue in chronic diseases such as obesity, type 2 diabetes, cancer and cardiovascular diseases. It is also a key feature of Nonalcoholic Fatty Liver Disease (NAFLD). The main aim of this study is to quantify the insulin levels as well as glucose, ALT and AST levels in the serum of obese NAFLD patients. The data obtained in this experiment will be used in future studies for sample stratification. The Cholestech LDX diagnostic tool was used in this experiment. Lipid Profile GLU and ALT.AST (GPT.GOT) cassettes were placed in the Alere Cholestech LDX system. The correlation analysis was performed for 70 serum samples of obese NAFLD patients in which 54 serum samples were obtained from ELISA technique and 16 serum samples were done in Cholestech LDX system. The significant values for TRG was 0.001 with correlation coefficient (0.397), HDL was 0.018 with correlation coefficient (-2.90), LDL was 0.107 with correlation coefficient (0.210), Non-HDL was 0.020 with

correlation coefficient (.285), TC/HDL was 0.021 with correlation coefficient (0.283) and GLU was 0.00 with correlation coefficient (0.499). The data obtained through correlation analysis and linear regression supports the main idea of this study. The increased level of IL-8, IL-6, TNFa and C-peptide insulin are found in the patients with higher glucose, ALT and AST levels.

### Natural History and Biodiversity

FREE-LIVING SOIL NEMATODE POPULATION DIVERSITY DYNAMICS AT AN *ASIMINA TILOBA* SITE IN VIRGINIA. Sarah R. Marzec & Theresa M. Grana, Department of Biological Sciences, Univ. of Mary Washington, Fredericksburg VA, 22401. Nematodes are microscopic roundworms that are highly successful in many environments. The model organism *Caenorhabditis elegans* is specifically a free-living nematode which can be found in soil but has been mostly isolated from anthropogenic habitats. Little is known about the environmental factors that affect *C. elegans* and where it is proliferate in nature. Information on ecological factors affecting *Caenorhabditis* species will be useful in identifying selective pressures that can influence genomic changes. The goals of this study are to find *C. elegans* and other *Caenorhabditis* species and shed light on relationships between the ecological factors and proliferating populations of nematodes. An *Asimina tiloba* site provides a natural Virginian habitat with a food source for *Caenorhabditis* species. This site is sampled every two weeks and any relevant ecological factors are recorded for the duration of a year. Nematodes are isolated from each soil sample and are separated based on morphology. The life stages of the nematodes from each sample are recorded and then the nematodes are sequenced for species identification. At present, five samples have been collected and several strains of nematodes have been isolated among and within the samples. All nematodes have shown to be in the dauer life-stage, a non-feeding migratory stage, showing that there are no established colonies. This is most likely due to the cold weather and we expect future results taken between the months of May through October to show proliferating populations.

INVENTORY AND CONSERVATION ASSESSMENT OF THE MOTH FAUNA OF VIRGINIA (LEPIDOPTERA). Steven M. Roble, Virginia Department of Conservation and Recreation, Division of Natural Heritage, Richmond, VA 23219. The insect order Lepidoptera includes the butterflies, skippers, and moths. Virginia's butterfly and skipper fauna of some 170 species has been well-studied for more than a century and continues to attract interest from biologists and amateur naturalists alike. In contrast, the much larger moth fauna of the Commonwealth remains poorly studied with the exception of a few pest species such as the Gypsy moth (*Lymantria dispar*). No formal compilation of the Virginia moth fauna exists, although survey efforts by staff of the Virginia Department of Conservation and Recreation, Division of Natural Heritage (DCR-DNH), during the past quarter century have begun to elucidate the composition, distribution, and conservation status of the "macro-moth" component of this fauna. Nearly 1,200 species of macromoths have been documented in Virginia, with a comparable number of "micro-moths" also expected to occur in the state, for a total

fauna of about 2,400-2,500 species. A number of these species have not been formally described in the scientific literature. Currently, the DCR-DNH rare animal list includes 95 species of moths (92 macro-moths and 3 micro-moths), 11 of which have not been recorded in the state in more than 50 years. None of these species is formally listed as threatened or endangered in Virginia. The DCR-DNH animal “watchlist” contains 123 additional species of macro-moths that may warrant conservation attention. With funding via a State Wildlife Grant from the Virginia Department of Game and Inland Fisheries and the U.S. Fish and Wildlife Service, DCR-DNH has recently developed a web-based atlas of Virginia’s rare Lepidoptera (butterflies, skippers, and moths) and Odonata (dragonflies and damselflies) that will debut in June 2013 at <http://www.vararespecies.org>.

DEVELOPMENTAL TOLERANCE TO NICOTINE DIFFERS BETWEEN INCIPIENT SPECIES OF A PARADITIC WASP WITH RESPECT TO HOST FOOD-PLANT. Justin P. Bredlau & Karen M. Kester, Dept. of Biology, Virginia Commonwealth University, Richmond VA 23284. Nicotine evolved as a defense against insect herbivores. In response, tobacco-feeding insects as well as parasitic wasps that attack and develop within tobacco-feeding insects evolved counter defenses to nicotine. We tested the hypothesis that two incipient species of the braconid wasp, *Cotesia congregata* Say, and their reciprocal hybrids would differ in developmental responses to nicotine dosage level in the host diet. “MsT wasps” originated from the solanaceous specialist, *Manduca sexta* L. (“tobacco hornworm”) on tobacco, and “CcC wasps” from the catalpa specialist, *Ceratomia catalpae* Boisduval (“catalpa sphinx”) on catalpa. Reciprocal crosses (MsT $\sigma$  x CcC $\varphi$ , CcC $\sigma$  x Ms $\varphi$ ) were established by pairing unmated males and females. Females were permitted a single oviposition into a 3<sup>rd</sup>-instar larva of *M. sexta*. Parasitized caterpillars were fed on a laboratory diet with 0%, 0.1%, or 0.3% (wet weight) nicotine until wasp larvae egressed from the host and spun cocoons. For each hornworm host, we counted the number of egressed and unegressed larvae, cocoons, and emergent adult wasps. Results demonstrate that MsT wasps are highly adapted to nicotine and responded to nicotine in a dosage-dependent manner. In contrast, exposure to 0.1% nicotine resulted in high mortality of CcC wasps. Responses of the hybrid crosses varied with respect to maternal wasp type (MsT or CcC) and sex. For example, most haploid males with CcC mothers failed to egress, whereas hybrid females from either cross did, suggesting that nicotine tolerance is a dominant heritable trait or suite of traits.

HABITAT CHARACTERISTICS INFLUENCING HIBERNATION SITE SELECTION BY WOOD TURTLES (*GLYPTEMYS INSCULPTA* LE CONTE), A THREATENED SPECIES IN THE SHENANDOAH VALLEY, VIRGINIA. J. A. Miller & W. S. Bousquet, Env'tl. Studies Program, Shenandoah Univ., Winchester VA 22601 & T. S. B. Akre, Dept. of Bio. Longwood Univ., Farmville VA 23909. In November 2012, researchers captured hibernating wood turtles, *Glyptemys insculpta* Le Conte, and recorded habitat data twice along two stream reaches of a tributary to the Shenandoah River. Hand capture techniques followed the standardized protocol used by the Wood Turtle Study Group, which required three researchers to search for turtles in 50-meter segments of 1.0-Kilometer stream reaches. Stream habitat variables for each 50-meter segment and descriptive data for each turtle captured were recorded.

Most turtles occurred within 5 to 10 meters of the stream banks, demonstrating preference for shallow, slow-moving stream segments with abundant leaf packs. Statistical analysis revealed a positive correlation between the number of root masses and the number of wood turtles ( $r = 0.268$ ). Stream depth and number of turtles showed a negative correlation ( $r = -0.215$ ). Root masses had a stronger correlation at the first site ( $r = 0.428$ ) than at the second site ( $r = -0.029$ ). Although the first site and overall creek data showed low correlations between stream width and number of turtles found, the second site exhibited a high correlation ( $r = 0.5$ ). Further study at these and other sites in the Shenandoah Valley region will help identify the stream variables that have the greatest influence on wood turtle hibernation site selection, allowing priorities to be set for stream protection and rehabilitation.

DEPOSITIONAL HISTORY OF THE CARMEL CHURCH BONEBED, A MIOCENE (14 Ma) MARINE VERTEBRATE SITE IN CAROLINE COUNTY, VIRGINIA. Alton C. Dooley, Jr., VA Museum of Natural History, Martinsville, VA 24112. The Carmel Church Quarry in eastern Virginia includes at least eight disconformity-bound Tertiary marine units. One of these units contains a dense fossil vertebrate bonebed, consisting primarily of cetaceans, chondrichthyans, osteichthyans, and turtles, with smaller numbers of other marine and terrestrial mammals. Previous studies of terrestrial mammals and diatoms indicate that the bonebed correlates to the uppermost part (Bed 15) of the Plum Point Member of the Calvert Formation, with an age of 13.7-14.7 Ma (middle Miocene), and that it disconformably overlies the early Eocene Nanjemoy Formation. Yet the bonebed contains large numbers of reworked fossils, including taxa with little or no temporal overlap, such as *Carcharocles megalodon*, *Carcharocles* cf. *C. angustidens*, and *Otodus obliquus*. Most reworked fossils in the bonebed show extensive evidence of abrasion, but some Eocene taxa (such as *O. obliquus*) are occasionally found in pristine condition. The presence of these specimens suggests that the Nanjemoy-Calvert disconformity included at least two transgressions between the late Eocene and middle Miocene that were completely removed by erosion prior to deposition of the Calvert Formation, and that pristine Eocene fossils have been reworked from the Nanjemoy into the Calvert through bioturbation.

#### Posters

HABITAT USE AND SWIMMING BEHAVIOR OF LONGNOSE DACE (*RHINICHTHYS CATARACTAE*) FROM A MERCURY CONTAMINATED RIVER. Kimberly J. Bolyard & Joelle E. Bennett, Dept. of Biol., Bridgewater College, Bridgewater VA 22812. Mercury in the environment has the potential to negatively affect the behavior of aquatic organisms through impacts on the nervous system and other physiological functions. We investigated the habitat use and swimming performance of longnose dace (*Rhinichthys cataractae*) from a river with mercury contamination and from a non-contaminated river in the Shenandoah Valley. Laboratory studies showed that dace from the non-mercury river spent significantly more time in areas containing large sticks than in areas with pine brush, pallet wood, or nothing. Dace from the mercury contaminated river did not spend significantly more time in any particular section of the test pool. In a second test, dace from the mercury

contaminated river spent more time swimming in a current over a five minute test period than did dace from the uncontaminated river but the difference was not significant. Finally, tested fish from the uncontaminated river were significantly longer than fish from the mercury contaminated river though they were not significantly heavier. As a result of spending less time in cover and possibly more time swimming in a current, longnose dace in mercury contaminated rivers may experience higher risk of predation than dace in uncontaminated rivers.

DESCRIBING NEMATODE DIVERSITY. Jessica A. Dochney, Thy N. Mai, & Theresa M. Grana, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Nematodes play important roles in nutrient cycling and decomposition. The nematode phylum is one of the most diverse and abundant groups of animals, with a projected 1 million species. However, fewer than 29,000 nematode species have been described and only a few species have been studied in laboratories. The nematode, *Caenorhabditis elegans* has been well studied as a genetic and developmental model organism. Such studies have contributed to our understanding of basic cell biology, genetics, and neurobiology. Our goal is to find and describe new species of nematodes, particularly those that could build upon our understanding of *Caenorhabditids*. Since 2005, the number *Caenorhabditids* known to researchers has doubled from 22 to nearly 50. A representative of one of these new species was found on the UMW campus on the northwest side of Seacobeck Hall. Our work is part of the formal description of this new species, which will help other researchers in understanding the life history of *Caenorhabditids*. To describe the new species, we are taking high-resolution images, measuring identifying body features, and comparing these data to described species.

LEARNED RESPONSES TO HERBIVORE-INDUCED PHYTOCHEMICALS IN THE PARASITIC WASP, *COTESIA CONGREGATA* (SAY) (HYMENOPTERA: BRACONIDAE). Christopher Crockett & Karen M. Kester, Dept. of Biology, Virginia Commonwealth University, Richmond VA 23284. Volatile phytochemicals, produced in response to herbivore feeding, aid parasitic wasps in the location of suitable herbivore hosts for reproduction. Several studies have demonstrated that wasps learn these host-induced volatiles (HIVs) in association with oviposition ("ovipositional learning") but whether wasps also can learn these cues within a critical period following adult emergence ("post-emergence learning") is not known. We tested the hypothesis that *Cotesia congregata*, an endoparasitoid of the tobacco hornworm (*Manduca sexta* L.) learns to recognize HIVs from tomato (var. 'Castlemart') through post-emergence learning. Inexpensive two- and four-way choice olfactometers were built and evaluated for use with this species, and a behavioral assay was developed. To test our hypothesis, newly-emerged wasps were given a post-emergence experience with leaves from a plant on which a hornworm had fed ("induced leaves") or a control plant ("non-induced leaves"). After a refractory period of 48 hours, orientation preferences of wasps to induced or non-induced leaves were assayed using a two-way T-tube olfactometer. More wasps experienced with non-induced leaves chose volatiles from non-induced leaves ( $X^2 = 3.92$ ,  $df=1$ ,  $N = 50$ ,  $p < .0477$ ), and more wasps experienced with induced leaves chose volatiles from induced leaves ( $X^2 = 6.48$ ,  $df=1$ ,  $N = 50$ ,  $p < .0109$ ). Results suggest that parasitic wasps can learn HIVs though post-

emergence experience. Continuing work will compare wasp responses to 'Castlemart' mutants with two levels of HIV silencing.

MIGRATION DYNAMICS OF NORTHERN SAW-WHET OWLS IN THE INNER PIEDMONT OF CENTRAL VIRGINIA. Esther Stine & Gene D. Sattler, Dept. of Biology & Chemistry, Liberty Univ., Lynchburg, VA 24502. Northern Saw-whet Owls (*Aegolius acadicus*) were mist netted during fall migration in Campbell County, Virginia in 2002-2007 and in 2012 using an audiolure of the male advertisement call. Birds were aged using feather molt criteria and sexed using a wing-mass discriminate function. The number of owl captured ranged from a low of 7 to a high of 101. The majority of birds were encountered during the first two weeks of November, with a median capture date of November 9<sup>th</sup>. In most years adults predominated, making up at least 68% of captures. However, both 2007 and 2012 were invasion years in which higher than normal numbers were netted and hatch year birds predominated (59% in both years). Females predominated in each year, making up at least 61% of captures. The inner Piedmont of central Virginia was established as an important corridor for Northern Saw-whet Owl migration, and capture rates exceeding those found on the Coastal Plain where the species' migration in Virginia has previously been investigated.

AGE AND PALEO-ENVIRONMENTAL SIGNIFICANCE OF UPPER PALEOZOIC OSTRACODES FROM THE APPALACHIAN AND EASTERN ILLINOIS BASINS. Shelby C. Sanders & Neil E. Tibert, Department of Earth and Environmental Sciences, University of Mary Washington, Fredericksburg, VA 22401. Ostracodes recovered from eastern North American coal measures in the Appalachian and Illinois Basins have the potential to resolve the temporal and spatial relationships of the strata deposited during the Pennsylvanian-Permian boundary interval. The Greene Formation of the Dunkard Group is the youngest Paleozoic stratigraphic unit in the Appalachian Basin that contains plant fossils of uncertain age. Fossil ostracodes from the GIL 30 core in western Kentucky (Illinois Basin) are comparable to the nonmarine ostracode assemblages from the Dunkard and can be used to constrain the age of the youngest sedimentary rocks in the Appalachian Basin. The GIL30 core was recovered from a down-faulted block located near Sturgis, KY and contains strata deposited during the latest Pennsylvanian and earliest Permian. We observed three ostracode assemblages in the GIL 30 core: (A) a marine assemblage dominated by *Hollinella cushmani*; (B) a brackish assemblage dominated by *Geisina upsoni*, *Cavellina nebrascensis*, and *Velatomorpha fittsi*; and (C) a freshwater assemblage dominated by *Whipplella cuneiformis* and *Haworthina bulletta*. The freshwater assemblage in the GIL 30 can be correlated with confidence to the uppermost units of the Greene Formation (e.g., Windy Gap Limestone) of the Dunkard Group in the stratotype area of northeastern West Virginia and southwestern Pennsylvania. These findings confirm an Early Permian age for at least the uppermost Dunkard Group strata.

TURTLES OF THE FREDERICKSBURG CANAL: INTRODUCED SPECIES AND ESTIMATES OF POPULATION SIZES. Y. Takeda & W. Wieland, Department of Biological Sciences, Univ. of Mary Washington. The yellow-bellied slider (*Trachemys scripta scripta*), a non-native to the Fredericksburg area, were found in the



Fredericksburg canal in the spring of 2011. The objectives for summer 2012 was to determine if this species has established a population, and in addition which species of turtles live in the canal and obtain life history information on these species, including estimates of population size. Hoop nets were placed along a 150 m stretch of the canal in the general area of where the single specimen of the yellow-bellied slider was found. Length and weight were taken for each individual and we attempted to determine age by counting the ridges on the plastron. Population size was determined using open population mark-recapture models. Turtles captured in the canal were red-bellied cooters (*Pseudemys rubriventris*), painted turtles (*Chrysemys picta*), eastern snapping turtles (*Chelydra serpentina serpentina*), stinkpots (*Sternotherus odoratus*), and the red-eared slider (*Trachemys scripta elegans*). The yellow-bellied slider was not found. The single specimen found in 2011 was likely introduced as these are common in the pet trade. However, the red-eared slider is also a non-native. The population size estimates of species for which we had a sufficient sample size are given by the Jolly-Seber model (open population) for the area of focus (roughly 150m). Estimates include population size and 95% confidence interval: red-bellied cooters, 35 (24-74); painted turtles, 9 (9-12); red-eared slider, 23 (10-114). Age determination proved to be problematic. Estimates for growth rates were determined for red-bellied cooters. However, due to small sample size and the short time span over which the study was conducted (29 capture days) these estimates are suspect.

## Psychology

STORIES OF BYSTANDER INTERVENTION FROM ACROSS THE GLOBE. S.Y. Teie, P. Randall, R. Wallace & B. Rivero, Center for Applied Behavior Systems, Department of Psychology, Virginia Tech, Blacksburg VA 24060. Actively Caring for People (AC4P) wristbands are used to recognize others for performing prosocial behavior. In 2011, the website AC4P.org was launched to provide a public space for individuals to share their experiences around AC4P wristband recognition. 74.4% of wristbands were passed by beneficiaries of kind acts who reciprocated with the wristband (Reciprocators), and 24.6% of wristbands were passed by observers of kind acts who intervened with the wristband (Bystanders). The qualitative analysis of AC4P stories revealed a differentiation between stories about wristbands passed by Reciprocators and those passed by Bystanders. Future research will explore gender interactions using larger sample sizes.

A BEHAVIOR-BASED INTERVENTION TO INCREASE PROSOCIAL RECOGNITION IN AN ORGANIZATION. K. M. Pacque, S. M. McCarty, S. Butterworth & C. Holmes, Department of Psychology, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Today's fraternal culture needs more caring, compassionate, and interpersonal relationships. News reports document problems from binge drinking and sexual assault charges, to the death of organization members from hazing. 73 students in a Greek fraternity received an Actively Caring for People (AC4P) intervention and used Twitter to recognize prosocial behavior. Students self-reported frequency data and relational data on behaviors and relational ties, respectively. Social network analysis (SNA) and multiple regression analyses assessed

influence ties, power ties, influence centrality, and power centrality on performance of recognition. Additionally, in- and out-degree ties of Aristotle's three variations of friendship predicted organization members' sense of organizational belonging to the fraternity. In general, multiple regression models with SNA measures predicted AC4P-related behaviors better than frequency ties alone. Only friendships based on "goodness" predicted students' sense of belonging in their organization.

**A BULLYING-PREVENTION INTERVENTION FOR MIDDLE SCHOOLS: PROMOTING AND REWARDING PROSOCIAL BEHAVIOR TO REDUCE AGGRESSION.** B. Tarzia, L. Anderson, G. Yam & M. Armstrong, Center for Applied Behavior Systems, Department of Psychology, Virginia Tech. This paper discusses the development of an Actively Caring for People (AC4P) approach to reduce bullying in middle schools. An intervention teaching character strengths enabling students to demonstrate discretionary, prosocial behavior is likely to have positive outcomes. An assessment was conducted to evaluate the relationship between identified AC4P character strengths and positive outcomes across two schools in Southwest, VA. Students were asked to score their subjective well-being, hope, gratitude, self-efficacy and self-esteem, as well as their involvement in bully-victim behavior and discretionary, prosocial behavior (i.e. AC4P behavior). Using regression analyses, students' self-reported scores in hope, gratitude and self-efficacy were analyzed. Hope and gratitude were significant predictors in whether or not they participated in bullying and/or victimizing behavior. In regards to AC4P behavior, hope was a significant predictor for both performing and receiving AC4P behavior. A one-way ANOVA was run with Tukey's post hoc breaking down students' bullying behavior by category (i.e. uninvolved students, bullies-only, victims-only and bully-victims). Students' self-reported "hope" scores were significantly different in all three bully-behavior categories (i.e. bullies-only, victims-only and bully-victims) from uninvolved students.

**THE EFFECT OF NEGATIVE AFFECT ON VISUAL SEARCH PERFORMANCE WITH AND WITHOUT AUTOMATION.** Clinton Carter, Rachel R. Phillips, & Poornima Madhavan, Department of Psychology, Old Dominion University, Norfolk VA 23529. To examine the impact of affective valence on threat detection performance with and without automation under different levels of uncertainty, participants completed a visual search task in which they had to identify foe presence or absence in synthetic aperture radar images. Participants consisted of 67 students (23 males, 44 females) between the ages of 18 and 51 ( $M = 21.27$ ,  $SD = 5.84$ ) from a large southeastern university. Participants were randomly assigned to one of two affective valence conditions (negative or neutral) and one of two automation conditions (no automation or perfect automation) and completed the visual search task with both easy and difficult images. A series of 2 automation x 2 affective valence x 2 task difficulty mixed factorial ANOVAs were conducted for the dependent variables of sensitivity, response bias, and confidence. Results revealed that there was a significant interaction between affective valence and automation for response bias. Specifically, in the negative condition, response biases were similar between those who received the assistance of the automation and those who did not. However, in the neutral condition, those who received the automation were more liberal than those who did not receive the automation. These participants also reported the highest confidence and were part

of the most sensitive group. These findings suggest that participants in the negative condition may have utilized the aid less than those in the neutral condition. This indicates that the potential emotional experiences of an operator should be considered when determining when and where to integrate automated decision aids.

HOW THE FRAMING OF METAPHORS AFFECTS PUBLIC POLICY TOWARD THE MENTALLY ILL. Victoria E. Bennett & Della N. Gibson, Department of Psychology, University of Mary Washington, Fredericksburg, VA 22401. Metaphors have been shown to influence people's decisions about campaigns, ads, and politics. Specifically, studies have looked at how metaphors have influenced attitudes toward crime and obesity. We examined the effect of blaming and victimization metaphors on public policy attitudes toward the mentally ill. It was hypothesized that victimization metaphors, as compared to blaming metaphors, would lead to increased support of outpatient treatment, increased support of government funding, and increased support of rights and opportunities for the mentally ill. The results showed no significant differences between the three conditions. Additionally, metaphors may not influence people with strongly held beliefs toward an issue.

A CONCEPTUAL MODEL FOR BYSTANDER INTERVENTION: EXPANDING PROSOCIAL BYSTANDER BEHAVIOR. M. Sihalath, B. Wright, P. Pierucci & R. Cobb-Ozanne, Center for Applied Behavior Systems, Department of Psychology, Virginia Tech, Blacksburg VA 24060. The frequency of bullying and interpersonal violence remains high throughout our culture. In these situations where intervention is needed, bystanders play a vital role in potentially reducing interpersonal violence. Bystanders in emergency or violent situations choose whether or not to intervene for the prevention of harm and/or injury. This extends the bystander intervention model from the prevention of interpersonal harm to the promotion of prosocial behavior. Participants were given green wristbands to recognize desirable prosocial behavior and were measured on the four factors from the Latané and Darley model (i.e., "notice", "interpret", "responsibility", and "skills"). In regards to predicting prosocial recognition, the results suggest "responsibility" and ability to "interpret" were significant predictors. In regards to predicting intention to intervene, the data suggests "notice" to be a significant predictor.

## Statistics

STATISTICAL PREDICTION FOR VIRGINIA LYME DISEASE EMERGENCE BASED ON SPATIAL-TEMPORAL COUNTS DATA. Yuanyuan Duan<sup>1</sup>, Jie Li<sup>1</sup>, Yili Hong<sup>1</sup>, Korine N. Kolivras<sup>2</sup>, James B. Campbell<sup>2</sup>, Stephen P. Prisley<sup>3</sup> & David N. Gaines<sup>4</sup>, <sup>1</sup>Department of Statistics, Virginia Tech, Blacksburg, VA 24061<sup>2</sup>, Department of Geography, Virginia Tech, Blacksburg, VA 24061, <sup>3</sup>Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA 24061 & <sup>4</sup>Virginia Department of Health, Richmond, Virginia 23219. The emergence of infectious diseases over the past several decades has highlighted the need to better understand and prepare for epidemics as endemic infectious diseases. These diseases are usually expanding their geographic range and are recorded over multiple time periods, making

the analysis and prediction more complicated. This study is based on areal (census tract level) counts data of lyme disease cases in Virginia from 2003 to 2010. Our visualization of incident rate using kernel smoothing showed the spatial-temporal emergence pattern of Lyme disease. We built a spatial Poisson regression model with random effect to incorporate spatial-temporal correlations. The random effects were modeled by Conditional Auto-Regressive (CAR) model. We used Markov chain Monte Carlo (MCMC) algorithm to fit the model in a Bayesian framework. Using this model, we analyzed 5 year sum counts from 2006 to 2010 to identify significant landscape/demographic variables for different eco-regions. We also analyzed yearly counts data and predict 2011's count based on previous years. This study was funded by NSF-BCS-GSS Grant.

PRODUCT COMPONENT GENEALOGY MODELING AND WARRANTY RETURN PREDICTION. Caleb King<sup>1</sup>, Yili Hong<sup>1</sup> & William Q. Meeker<sup>2</sup>, <sup>1</sup>Department of Statistics, Virginia Tech, Blacksburg, VA 24061 & <sup>2</sup>Department of Statistics, Iowa State University, Ames IA 50011. Many industrial products consist of multiple components that are necessary for maintaining their integrity. There is already an abundance of literature on modeling the lifetime of such components through competing risks models. However, none of these models take into consideration the fact that many of these components are part of a specific generation of components and that each of these components may go through several generations throughout a period of observation. We believe that by incorporating this extra information, which is readily available in most product warranty databases, better accuracy can be achieved in predicting time to failure thus yielding more reliable warranty return information. We present methods for estimating parameters for this new generational model as well as provide a comparison with existing methods through the use of simulation. Our results indicate that the generational model outperforms the existing methods in predicting warranty returns.

DEGRADATION DATA ANALYSIS USING NONLINEAR MODELS VIA SHAPE-RESTRICTED SPLINES. Zhibing Xu & Yili Hong, Department of Statistics, Virginia Tech, Blacksburg, VA 24061. The traditional reliability analysis is based on the failure-to-time data. However, many products are designed with high reliability and low risk for failures. It is hard to collect enough failure-to-time data to give a precise estimation for the highly reliable products in a limit time, even under accelerated conditions. Comparing to the traditional life time data, degradation data not only can provide enough information in a short time, but also give a better estimation and prediction of the life time of products. With the improvement of technology, life-affecting environmental variables are recorded as well as the degradation measure over time. For example, the large amount of ultraviolet exposure will accelerate the degradation of the polymer coating. Thus, it is important to incorporate the environmental variables, also called dynamic covariates, into the degradation path model. Existing research suggests a linear mixed effect model with splines to combine the dynamic covariates. With unspecific effect function for the dynamic covariates, shape restricted splines method is used. However, the linear model may not widely used in real applications, because most of the degradation data follow a nonlinear trend, especially in the pharmacokinetic research. In this paper, we propose a nonlinear mixed

effect model combining with dynamic covariates to model the degradation data. Shape restricted splines are used in the proposed model and a modified alternating algorithm is developed. The performance of the algorithm is evaluated by simulations. An outdoor weathering dataset is used for illustration of the proposed method.

DECISION TREES WITH HIGH DIMENSIONAL DATA AND EXPLICIT COVARIATE SELECTION. Lucas Roberts, Department of Statistics, Virginia Tech, Blacksburg, VA 24061. In this talk, we survey the decision tree literature from both a model based and a heuristic perspective. Primarily, these methods have been successfully deployed in cases where prediction is of interest. When we are mainly interested in inference, which in this context means identifying the most useful covariates, these methods can exhibit poor behavior. These inferential problems are exacerbated in high dimensional spaces. After reviewing some general covariate filtering approaches, we present our method, which does covariate filtering and decision tree search jointly. We state necessary and sufficient conditions ensuring consistency of the tree classifier and we show the benefits of our approach through simulated and real examples. We also give a rule for ranking and selecting covariates using output from our model. We compare our covariate ranking and selection procedure to current common approaches.

CLUSTER-BASED PROFILE MONITORING IN PHASE I ANALYSIS. Yajuan Chen, Jeffery B. Birch & William Woodall, Department of Statistics, Virginia Polytechnic Inst. & State Univ., Blacksburg VA 24061. An innovative profile monitoring methodology is introduced for Phase I analysis. The proposed technique, referred to as cluster-based profile monitoring, incorporates a cluster analysis phase to aid in determining the possible existence of "profiles" in the historical data set (HDS) resulting from an out-of-control process. To cluster the profiles, the proposed method first replaces the data from each sampled unit with an estimated profile, using some appropriate regression method, and clusters the profiles based on their estimated parameters. This cluster phase then yields an initial main cluster which contains at least half the profiles. The initial estimated population average (PA) profile is obtained by fitting a linear mixed model to those profiles in the main cluster. Profiles, determined using the Hotelling's  $T^2$  statistic, that are not contained in the initial main cluster are iteratively added to the main cluster and the mixed model is used to update the estimated parameters for the PA profile. Those profiles contained in the final main cluster are considered as resulting from the in-control process while those not included are considered as resulting from an out-of-control process. A simulated example and Monte Carlo results demonstrate the performance advantage of this proposed method over a current non-cluster-based method with respect to more accurate estimates of the PA parameters.

ESTIMATION AND EVALUATION OF OPTIMAL THRESHOLDS FOR THE BELIEVE THE POSITIVE SEQUENTIAL TESTING STRATEGY. Amber R. Wilk & Donna K. McClish, Department of Biostatistics, Virginia Commonwealth University, Richmond VA 23219. Many continuous medical tests rely on a threshold for diagnosis. A sequential testing strategy, called Believe the Positive, classifies a patient positive if either the first test is greater than a threshold  $\theta_1$  or negative on the

first test and greater than 0.2 on the second test. A threshold pair  $\theta = (\theta_1, \theta_2)$  was defined as optimal if it maximized  $GYI = \text{Sensitivity (Se)} + r * (\text{Specificity (Sp)} - 1)$ . Of interest is to determine if the optimal threshold pair estimates are “good” when calculated from a sample. Formulas were derived to estimate  $\theta^*$  assuming tests follow a binormal distribution, using the Newton-Raphson algorithm. A simulation study was performed assessing bias, root mean square error (RMSE), percentage of under/over estimation of Se/Sp, and coverage of simultaneous confidence intervals (SCI)/ellipses for sets of population parameters and sample sizes. Bootstrapping was used to estimate the variance of each optimal threshold pair. When the area under the curve (AUC) between the two tests is equal, the median RMSE and median bias in Se and p appears to decrease. The coverage of the SCI and confidence ellipses improves as sample size, AUC, and the ratio of standard deviations of each group increases. This also holds true for percentage of overestimation of Se and Sp except when the allocation between groups favors those without disease. This method is an improvement over an empirical estimate.

**LIKELIHOOD BASED FORECASTING OF LOCALLY CHANGING VARIANCE STRUCTURED TIME SERIES DATA.** Rajan Lamichhane & Norou Diawara, Dept. of Mathematics and Statistics, Old Dominion University, Norfolk VA 23529. In this talk, we assume several non-linear time series models with additive noise components, and the model fitting is proposed in two stages. The first stage identifies the density ratio using all the clusters information, without specifying any prior knowledge of the underlying distribution function. In the second stage, we partition the time series into consecutive non-overlapping intervals of quasi stationary increments where the coefficients shift from one stable regression relationship to a different one using breakpoints detection algorithm. These breakpoints are estimated by minimizing the likelihood from the residuals. We develop a method based on finite mixture of Gaussian distributions to forecast this type of partitioned data. The Expectation-Maximization (EM) algorithm, with initial values obtained from the empirical estimates; give the estimates of mixture distribution. We propose likelihood based method to identify the most probable value from a pool of past values for the forecasting.

**ESTIMATING EXPLAINED VARIATION FOR AN UNDERLYING LINEAR MODEL USING LOGISTIC REGRESSION.** D. Sharma, Department of Mathematics and Statistics, James Madison University, Harrisonburg, VA 22807. The coefficient of determinant, also known as the  $R^2$  statistic, is used to assess the extent of the strength of the relationship between a response and explanatory variables in a linear regression model. It measures the proportion of variation in the response variable explained by a set of independent variables. In many real life events, interest lies on modeling the relationship between a continuous response variable and a set of predictors. But in practice, the continuous dependent variable of interest may not be observable and is usually represented by its binary proxy. In such situations, logistic regression is a popular choice. There are many  $R^2$  type statistics proposed to measure of explained variation for logistic regression. The pseudo  $R^2$  measure ( $R_L^2$ ) stands out because of its intuitive interpretation and independence on the proportion of success in the sample. It, however, severely underestimates the proportion of explained

variation ( $P^2$ ) in the variable underlying the binary indicator of event occurrence. In this research we present a method for estimating the explained variation for the underlying linear model using the pseudo  $R^2$  statistics obtained from a logistic regression analysis.

STRUCTURED FUNCTIONAL ADDITIVE REGRESSION IN REPRODUCING KERNEL HILBERT SPACES. Hongxiao Zhu<sup>1</sup>, F. Yao<sup>2</sup> & H. H. Zhang<sup>3</sup>, <sup>1</sup>Department of Statistics, Virginia Tech, Blacksburg, VA 24061, <sup>2</sup>Dept. of Statistics, University of Toronto, & <sup>3</sup>University of Arizona. Functional additive models (FAMs) provide a flexible yet simple framework for regressions involving functional predictors. The utilization of data-driven basis in an additive rather than linear structure naturally extends the classical functional linear model. However, the critical issue of selecting nonlinear additive components has been less studied. In this work, we propose a new regularization framework for the structure estimation in the context of Reproducing Kernel Hilbert Spaces. The proposed approach takes advantage of the functional principal components which greatly facilitates the implementation and the theoretical analysis. The selection and estimation are achieved by penalized least squares using a penalty which encourages the sparse structure of the additive components. Theoretical properties such as the rate of convergence are investigated. The empirical performance is demonstrated through simulation studies and a real data application.

DICTIONARY LEARNING FOR GENETIC DATA IN RELATED INDIVIDUALS. Xiaowei Wu & H. Zhu, Department of Statistics, Virginia Tech, Blacksburg, VA 24061. Dictionary learning has been widely used for sparse representation and data restoration in computer vision and image analysis. However, its application in statistical genetics has not been fully explored. We consider the problem of learning the sparsity structure of genetic data in related individuals. We propose a nonparametric Bayesian method based on a latent beta process factor analysis model. The method can be used in association study with related individuals for multiple purposes, for example, genotype imputation, estimation of allele frequency or kinship coefficients. Simulation study shows good performance and advantages of the proposed method in comparison with some existing methods. We also apply our method to the analysis of the GAW 18 data set.

EVALUATING THE HEALTH EFFECT(S) OF ENVIRONMENTAL CHEMICALS AND NUTRITION: CONSIDERATIONS FOR HIGHLY CORRELATED DATA. Chris Gennings, Virginia Commonwealth University, Richmond VA 23298. Biomonitoring of environmental chemicals in human tissues and fluids has shown that all people, not just those working in or living near major pollution sources, carry a “body burden” of synthetic chemicals in their blood, fat, mother’s milk, semen, urine and breath. Despite increasing public concern regarding the potential health effects of pervasive and persistent environmental exposures, there are few, if any, recommendations for life style changes to mitigate such effects. Our research focus is a holistic evaluation of the impact of environmental chemicals on health effects including metabolic endpoints (e.g., obesity, hyperlipidemia) and hepatotoxicity and the role nutrition may play in potentially mitigating the impact of chemical exposures. As such, we are not focused on single chemicals or nutrients – instead, we are focused

on their potential combined effects. Standard statistical methods suffer from multicollinearity effects due to the complex correlation structure among the components. We have developed novel statistical methods to determine empirical weights that identify subsets of components most associated with a response variable. The results are weighted indices of body burden and nutrients – i.e., simple to understand and visualize indices that may be easily combined with other behavioral data for additional analyses. Characterization of the approach will be described through simulation studies. The methods will be demonstrated using NHANES data.

### Structural Biology, Biochemistry, and Biophysics

UBIQUITIN MODULATES TOLLIP'S LIPID-MEDIATED ENDOSOMAL MEMBRANE BINDING. S. Mitra, A. Traughber, S. Gomez, & D. G. S. Capelluto. Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. Ubiquitylation is a highly controlled post-translational modification of proteins, in which proteins are conjugated either with monoubiquitin or polyubiquitin chains. Ubiquitin modifications on target proteins are recognized by ubiquitin-binding domains, which are found in several effector proteins. In this study, we describe for the first time how ubiquitin controls the function of the Toll-interacting protein (Tollip), which is an effector protein in the innate immune signalling pathway and an adaptor protein for endosomal trafficking. We have demonstrated that the central C2 domain of Tollip preferentially interacts with phosphoinositides. Remarkably, we have observed an ubiquitin dose-dependent inhibition of binding of Tollip to phosphoinositides and it does so specifically by blocking Tollip C2 domain-phosphoinositide interactions. This led us to discover that the Tollip C2 domain is a novel ubiquitin-binding domain. In addition, we have biophysically characterized the association of the Tollip CUE domain to ubiquitin and compared it with Tollip C2 domain-ubiquitin binding. We have also found that ubiquitin binding to dimeric Tollip CUE domain induces a drastic conformational change in the protein, leading to the formation of a heterodimeric Tollip CUE-ubiquitin complex. These data suggest that ubiquitin binding to the Tollip C2 and CUE domain and ubiquitin-mediated dissociation of CUE dimer reduces the affinity of Tollip protein for endosomal phosphoinositides, allowing Tollip's cytoplasmic sequestration for its cytosolic commitments.

MECHANISTIC STUDIES OF A FLAVIN DEPENDENT LYSINE MONOOXYGENASE FROM *NOCARDIA FARCINICA*. R.M. Robinson, P. Rodriguez, N. Keul, & P. Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. The kinetic and chemical mechanism of the lysine monooxygenase Nocobactin G (NbtG) from *Nocardia farcinica* was investigated using both steady-state and rapid reaction kinetics. NbtG hydroxylates both L- and D-lysine, a unique characteristic among members of the *N*-hydroxylating monooxygenases (NMOs), which are typically selective for the L-stereoisomer of their respective substrate. NbtG is mostly uncoupled (~75%) and produces superoxide and hydrogen peroxide as oxidative by-products. O<sub>2</sub> consumption increases from 0.581 s<sup>-1</sup> in the absence of lysine to 1.09 s<sup>-1</sup> and 3.03 s<sup>-1</sup> when saturating concentrations of L- and D-



lysine are present, respectively. NbtG has a ~8-fold higher  $k_{cat}/K_m$  value for L-lysine suggesting it is the preferred substrate. Reduction of NbtG by NADPH is independent of both lysine stereoisomers and occurs in two exponential phases with rates of 1.00 s<sup>-1</sup> and 0.119 s<sup>-1</sup> for the first and second phase, respectively. Primary kinetic isotope effect studies with pro-(R)-NADPD indicate a  $^Dk_{red}$  value of ~1.5 where both phases of reduction are isotopically sensitive. The rate of flavin oxidation is affected by lysine and increases from  $3.1 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  in the absence of lysine to  $3.9 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  and  $5.4 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  when saturating concentrations of L- and D-lysine are present, respectively. Solvent kinetic isotope studies yield a  $^{D_2O}k_{cat}$  value of ~2.4. These results indicate the active site of NbtG is loosely stereospecific for lysine and hydride transfer is only partially rate-limiting, but a chemical step that is influenced by proton exchange with solvent is rate-limiting. This work was supported by a grant from the National Science Foundation MCB-1021384.

A CONSERVATIVE ISOLEUCINE TO LEUCINE MUTATION CAUSES MAJOR REARRANGEMENTS AND COLD-SENSITIVITY IN KLENQAQ DNA POLYMERASE. E. Y. Wu<sup>1</sup>, E. P. Kornberg<sup>1</sup>, B. Zielinski<sup>1</sup>, W. M. Barnes<sup>2</sup>, & M. B. Kermekchiev<sup>3</sup>, <sup>1</sup>Dept. of Biology, University of Richmond, Richmond, VA 23173, <sup>2</sup>Dept. of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, & <sup>3</sup>DNA Polymerase Technology, Inc., St. Louis, MO 63104. Assembly of PCRs at room temperature can lead to low yields or unintentional products due to mispriming. A conservative mutation of isoleucine 707 to leucine in DNA polymerase I from *Thermus aquaticus* substantially decreases its activity at room temperature without compromising its ability to amplify DNA. To understand why such a small change located 24Å from the active site can have a large impact on enzyme activity at low temperature, we solved the X-ray crystal structure of the large fragment of *Taq* DNA polymerase containing the cold-sensitive mutation (Cs3C KlenTaq) in the closed, ternary (E-DNA-ddNTP) and open, binary (E-DNA) complexes. The Cs3C KlenTaq ternary complex was identical to the wild-type except for the mutation and a rotamer change in a nearby phenylalanine 749, suggesting that the enzyme should remain active. However, soaking out the nucleotide substrate at low temperature results in an altered binary complex made possible by the rotamer change at F749 near the tip of the polymerase O-helix. Surprisingly, two adenosines in the 5'-template overhang fill the vacated active site by stacking with the primer strand, thereby blocking the active site at low temperature. These results explain the cold sensitivity phenotype of the I707L mutation in KlenTaq and serve as an example of a large conformational change affected by a conservative mutation.

EVOLUTION AND NETWORK ANALYSIS OF LIPOCALIN PROTEINS USING COMPUTATIONAL APPROACHES. Nardos F. Sori & Lesley H. Greene, Dept. of Chemistry and Biochemistry, Old Dominion University, Norfolk Va. 23529. The lipocalins are a functionally diverse and divergent superfamily of proteins that share a common three-dimensional structure which consists of an antiparallel β-barrel and a C-terminal α-helix. Lipocalins are found in various organisms with a wide range of functions such as pheromone activity, lipid transport and coloration. Even though lipocalins are found widely in nature, the first indication of its existence in plants was observed in late 20<sup>th</sup> century by the sequence analysis of Violaxanthin de-epoxidase

and Zeaxanthin epoxidase, which were shown to be lipocalin-like proteins. Recently lipocalins from Wheat and Arabidopsis were identified as true lipocalins through the elucidation of three structurally conserved regions. The study of these lipocalins is vital as these proteins are believed to help plants tolerate oxidative stress and extreme conditions which broadens our understanding of plant sustainability in different environments. Protein structure homology modeling was used to construct a preliminary structure of the temperature induced wheat lipocalin. Furthermore, conservation of amino acids through evolution was analyzed by using modified Shannon entropy parameter with 20 divergent lipocalins. It revealed that three positions have conservation that have greater than 0.45 which indicates high conservation.

**THERMOTOLERANCE IN MAMMALIAN CELLS.** Ovidiu Lipan, Dept. of Phys., Univ. of Richmond, Richmond VA, 23173. The heat-shock response network controls the adaptation and survival of the cell against environmental stress. A key element of the heat-shock network is the heat-shock transcription factor-1 (HSF1), which is transiently activated by elevated temperatures. The HSF1 homotrimers bind to the heat shock element on the DNA and control the expression of the hsp70 gene. The Hsp70 proteins protect cells from thermal stress. The modulation of the activity of the hsp70-promoter by the intensity of the input stress is thus critical for cell's survival. The promoter transient activity starts from a basal level and rapidly increases once the stress is applied, reaches a maximum level and attenuates slowly back to the basal level. The molecular construct used as a measure of the response to thermal stress is a Hsp70-GFP fusion gene transfected in Chinese hamster ovary (CHO) cells. The time profile of the GFP protein depends on the transient activity, of the heat shock system. The GFP time profile is recorded using flow cytometry measurements, which allows a quantitative measurement of the fluorescence of a large number of cells ( $10^4$ ). The GFP responses to one and two heat shocks were measured for 261 conditions of different temperatures and durations. We found that: (i) the response of the cell to two consecutive shocks depends on the order of the input shocks. This phenomenon is thermotolerance without recovery time in between shocks; (ii) the responses may be classified as mild or severe, depending on the temperature level and the duration of the heat shock and (iii) the response is highly sensitive to small variations in temperature.

**INHIBITION OF SIDEROPHORE BIOSYNTHESIS BY TARGETING *A. FUMIGATUS* ORNITHINE HYDROXYLASE: A STRUCTURE-BASED VIRTUAL SCREENING STUDY.** S. Badicyan<sup>1</sup> & P. Sobrado<sup>1,2</sup>, <sup>1</sup>Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061 & <sup>2</sup>Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061. Siderophore A (SidA) from *Aspergillus fumigatus* is a flavin-dependent monooxygenase that catalyses hydroxylation of L-ornithine. N5-hydroxyl-ornithine is subsequently incorporated into hydroxamate-containing siderophores. The pathogenicity of *A. fumigatus* in mammals is dependent on the availability and function of siderophores. Thus, inhibition of the siderophore biosynthetic pathway may significantly affect the virulence of this fungus. SidA plays a central role in this pathway and is considered the key target for inhibitory study. Availability of several high-quality structures of SidA in complex with different ligands allowed the in-silico screening of a large library of drugs, natural products, and synthetic compounds. The screening results were sorted by the absolute and normalized

binding energies and then filtered based on the formation of at least one hydrogen bond to the part of the ornithine binding site with several hydrogen bond donors. The top hits have average molecular weights of 200 Da and  $\Delta G$  bindings of -7.5 kcal/mol. They are highly soluble nitrogen- or oxygen-rich compounds that make several hydrogen bonds with residues in the active site of SidA. This study was supported by NSF grant MCB 1021384 and by the Virginia Tech Biodesign and Bioprocessing Center.

A NOVEL TANK-BINDING KINASE 1 (TBK1) SUBSTRATE AND ITS ENDOGENOUS CONTROL OF A CRITICAL CATALYTIC HUB IN THE TYPE-I INTERFERON RESPONSE. James D. Marion, Jr. & Jessica K. Bell. Virginia Commonwealth University, Richmond, VA 23298. To defend against pathogen challenge, multi-cellular organisms mount an immune response that recognizes, sequesters and eradicates invading infectious agents. In response to receptor-mediated pathogen detection, several signaling pathways converge to activate TBK1, a kinase which phosphorylates substrates that lead to the production of pro-inflammatory cytokines and type-I interferons. Increasingly, TBK1 dysregulation has been linked to autoimmune disorders and cancers, heightening the need to understand its regulatory controls. Here, we describe the mechanism by which Suppressor of IKK $\epsilon$  (SIKE) inhibits the TBK1-mediated phosphorylation of IRF3, a transcription factor essential to type-I interferon production. Kinetic analysis revealed that SIKE not only inhibits IRF3 phosphorylation, but is also a high-affinity, TBK1 substrate. Further analysis determined that TBK1 phosphorylation of IRF3 and SIKE displayed negative cooperativity. Biophysical experiments revealed that TBK1 directly phosphorylated SIKE on 6 C-terminal residues that mediated TBK1:SIKE associations and exhibited striking homology to the IRF3 phosphorylation motif. Taken together, our findings demonstrate that SIKE functions as a TBK1 substrate that regulates this critical catalytic hub, not through direct repression of activity, but by redirection of catalysis through substrate affinity.

MOLECULAR MECHANISM OF MEMBRANE TARGETING BY ENDOSOMAL ADAPTOR PROTEINS. Shuyan Xiao & Daniel G. S. Capelluto, Protein Signaling Domains laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg VA, 24061. The Toll-interacting protein (Tollip) is an effector protein in the innate immune signaling pathway and an adaptor protein for endosomal trafficking. Tollip mediates protein sorting by association with Tom1, polyubiquitinated cargo, and clathrin. Tollip is modular in architecture with an N-terminal Tom1-binding domain (TBD), a central C2 domain, followed by a C-terminal CUE domain. Tom1 is a member of a protein family characterized by the presence of the VHS (Vps27/Hrs/STAM) domain at the N terminus, and a central GAT domain. In this study, we identified that Tollip-TBD directly binds to Tom1-GAT with a very high affinity. The solution structure of the complex formed by the Tollip-TBD and Tom1-GAT domain reveals that Tollip-TBD undergoes a coil/helix folding transition upon binding to GAT domain, forming an anti-parallel  $\beta$ -sheet followed by a short  $\alpha$  helix structure. The amphipathic helix  $\alpha$ 1 and  $\alpha$ 2 of GAT play a key role in the interacting with TBD. We also demonstrated that Tom1 GAT binds to Tollip TBD and ubiquitin in a mutually exclusive manner. Overall, our study provides the structural and molecular basis of Tollip-TBD and Tom1-GAT binding interactions, which will aid in

understanding the role of Tollip in endosomal trafficking. This work was supported by the American Heart Association.

**STRUCTURAL BASIS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP2) RECOGNITION BY THE TIRAP PIP2-BINDING MOTIF.** Xiaolin Zhao, Shuyan Xiao, Ayana Stukes & Daniel G. S. Capelluto, Department of Biological Sciences, Virginia Tech., Blacksburg VA. 24061. TIRAP is an adaptor molecule essential to the TLR2 and TLR4 signaling pathways. TIRAP contains a TIR domain, which is responsible for association with another adaptor protein, MyD88; and a N-terminal PIP2 binding domain (PBD) that binds to plasma membrane PIP2. The association of MyD88 and TLR is stabilized by the binding of TIRAP to the plasma membrane. Remarkably, a minimal region (residues 15-35) within PBD is sufficient to associate to the plasma membrane. We show that this region, that we called the PIP2-binding motif (PBM), adopts a helical conformation in the presence of the membrane mimic dodecylphosphocholine micelles and, more importantly, binds PIP2. NMR data indicates that TIRAP PBM binds PIP2 in a fast exchange regime, suggesting a moderate affinity for lipid binding. According to the TIRAP PBM resonance assignments, we found that conserved residues Lys16, Leu18, Lys20, Lys31, and Lys32 play important roles in association with PIP2. We are in the process to precisely identify the PIP2-binding site in TIRAP PBM and to obtain the solution structure of the peptide to understand the progressive mechanism and regulation of membrane insertion of the protein.

**PROBING CONFORMATIONAL CHANGES WITH MULTIWAVELENGTH COLLISIONAL QUENCHING.** Ellis Bell & Chun Li, Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173. To probe the local environments of tryptophans in proteins we have developed a novel analysis of fluorescence collisional quenching using multiple emission wavelengths, combined with differently charged quenchers (acrylamide: neutral, Iodide: negative & Cesium: positive). With N Acetyl tryptophan, similar Stern-Volmer constants are obtained at all wavelengths, as expected. With glutamate dehydrogenase, (4 tryptophans per subunit), 4 distinct families of constants are obtained with the highest occurring at long wavelengths and progressively decreasing as the wavelength band analyzed decreases showing this approach separates the effects on individual tryptophans in the protein. We have explored nucleotide effects on accessibility and charge environment of the 4 tryptophans. Results indicate that overall, nucleotide ligands NADH, NADPH and ADP significantly lower accessibility particularly in the region of subunit interfaces while GTP has less effect and in the case of W72 and W281 increases accessibility. Acrylamide quenching gives Stern-Volmer plots concave upwards, which become linear when lifetime measurements are used. With NAD(P)H, the charged quenchers, Iodide and Cesium (but not the neutral Acrylamide) give negative slopes which appear to result from differential effects on tryptophan-NAD(P)H resonance energy transfer. Funded in part by NSF Grant MCB 0448905 to EB.

**SYNTHETIC BIOLOGY: SYNTHETIC CIRCUITS AS A TOOL FOR BIMOLECULAR SYSTEMS EXPLORATION.** Warren C. Ruder, Dept. of Biological

Systems Engineering, Virginia Tech, Blacksburg VA, 24061. Professor Ruder joined Virginia Tech's Biological Systems Engineering department as an assistant professor in 2012. His expertise in synthetic biology, cellular biomechanics, and lab-on-a-chip systems complements Virginia Tech's strengths in biomolecular, biomedical, and environmental engineering. Most recently, he spent two and a half years in the synthetic biology laboratory of James Collins, a Howard Hughes Medical Investigator and NAE and IOM member based at Boston University and the Harvard Wyss Institute for Biologically-Inspired Engineering. Professor Ruder began his academic career studying civil and environmental engineering at MIT and graduated with an S.B. in 2002 with a focus in mechanics. He spent two years as a research assistant at Harvard Medical School studying cell calcium and cAMP signaling systems. He then spent four years at Carnegie Mellon University, where he received an M.S. in Mechanical Engineering and a Ph.D. in Biomedical Engineering. His thesis work focused on the design of micromechanical systems to explore cell biomechanics.

### Posters

WHERE DID THE SYNNUCLEINS COME FROM? A COMPUTATIONAL LOOK INTO THE PAST. Agatha Munyanyi, John Bedford & Lesley H. Greene, Department of Chemistry & Biochemistry, Old Dominion University, Norfolk VA 23529.  $\alpha$ -,  $\beta$ - and  $\gamma$ -Synucleins have been described as physiologically unstructured proteins primarily expressed in the brain tissue of vertebrates. Very recently  $\alpha$ -synuclein has been shown to form a helical tetramer which is resistant to fibril formation under physiological conditions. This folded form of  $\alpha$ -synuclein may be its natural state. A bioinformatics investigation was conducted to construct a synuclein superfamily to facilitate understanding their physiological structure, function and evolutionary history. The results identified similarity to an endoglucanase enzyme from bacterium *Acetobacter pomorum*, a CRE-DUR-1 protein from a nematode and a putative protein from the Tasmanian Devil. The Tasmanian Devil protein in turn identified a cytochrome c from the bacterium *Vibrio cholera*. In conclusion however, the synucleins most surprisingly seem to be orphan proteins in the vertebrate line.

MOLECULAR DOCKING AND VIRTUAL SCREENING OF PPAR- $\delta$  AGONISTS. K. E. Ascetta, S. N. Lewis & D. R. Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. Peroxisome proliferator-activated receptor subtype delta (PPAR- $\delta$ ) is a transcription factor that causes increased fatty acid transport and oxidation in white adipose tissue and skeletal muscle. It also plays a role in carbohydrate catabolism and fat synthesis in the liver. All of these processes require activation by agonists, which can be developed as treatments for metabolic disorders like type II diabetes and heart disease. One way to identify viable PPAR- $\delta$  agonists is through virtual screening. Screening requires a 3D model that best represents the receptor. In this study eighteen ligand-bound PPAR- $\delta$  crystal structures were tested with docking to determine which ones could serve as representative models. The fitness of these structures was evaluated by measuring root mean squared deviation (RMSD) and assessing protein-ligand interactions that match crystal structure references. The four fittest models were then used to screen a small group of published known PPAR- $\delta$  agonists that are not present in crystal structures. Here, only protein-ligand interactions

were used as measures for successful docking. Of the fittest models, 3SP9 and 1GWX fared the best in re-docking and cross-docking, while 3DY6 and 3SP9 most accurately predicted the protein-ligand interactions of the non- crystalized agonists. Presently, 3SP9 appears to be the model best fit for virtual screening to identify PPAR- $\delta$  agonists.

VIRGINIA TECH CENTER FOR DRUG DISCOVERY SCREENING LABORATORY. P. Sobrado<sup>1</sup>, N. J. Vogelaar<sup>1</sup> & D. G. I. Kingston<sup>2</sup>, <sup>1</sup>Department of Biochemistry, <sup>2</sup>Department of Chemistry, & <sup>1,2</sup>Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061. A new high-throughput screening laboratory has been established at Virginia Tech and is available for use by the scientific community. The facility instrumentation for microplate preparation includes a versatile liquid dispenser and a robot capable of high-accuracy plate-to-plate transfers. Detection of assay results can be done with UV-Vis absorbance, fluorescence, time-resolved fluorescence, glow luminescence, or through use of a UPLC. The facility has over 35,000 compounds available for screening use. The application of high-throughput screening methodologies was demonstrated in an assay designed to identify inhibitors of SidA (*A/SidA*) from *Aspergillus fumigatus*. In a preliminary screening of 640 compounds from the ChemBridge Diverset compound library, N-(5-chloro-2-methylphenyl)-N-[(4-methylphenyl)sulfonyl]glycine was identified as a potential inhibitor and found to have a SidA-binding constant of 14  $\mu$ M. Further screening of the ChemBridge library is underway. The Virginia Tech Center for Drug Discovery is supported by the Fralin Life Science Institute, the Virginia Tech College of Science, and the Virginia Tech College of Agricultural and Life Sciences.

CHARACTERIZATION OF A FLAVIN-DEPENDENT –MONOOXYGENASE FROM *CUPRIAVIDUS TAIWANENSIS*. Aaron Gringer<sup>1</sup>, Isabel Da Fonseca<sup>1</sup> & Pablo Sobrado<sup>1,2,3</sup>, <sup>1</sup>Department of Biochemistry, <sup>2</sup>Virginia Tech Center for Drug Discovery & <sup>3</sup>Fralin Life Science Institute, Virginia Tech, Blacksburg, VA 24061. Microbial iron acquisition depends on complex steps and many of which are not shared by higher eukaryotes. Inhibition of siderophore biosynthesis, a specific set of microbial reactions critical for iron acquisition and storage, is therefore a potential antimicrobial strategy. One essential enzyme for this process is a flavin-dependent N-monooxygenase (NMO). Seeking for more insights of how this enzyme class works, we cloned, purified and initially characterized the L-ornithine-5-monooxygenase from *Cupriavidus taiwanensis*. The codon optimized gene was cloned into pVP56K and expressed in *E. coli* BL21(DE3). The recombinant target protein with MBP fusion was purified using affinity column and digested to remove MBP by treatment with Tev protease. The  $k_{cat}$  and  $K_M$  values with L-ornithine are  $0.382 \pm 0.009 \text{ s}^{-1}$  and  $55 \pm 7 \mu\text{M}$ , and with NADPH are  $0.30 \pm 0.04 \text{ s}^{-1}$  and  $46 \pm 2 \mu\text{M}$ , respectively. Supported by NSF grant MCB 1021384.

COMPUTATIONALLY CHARACTERIZING THE STRUCTURAL DYNAMICS AND INTERACTIONS OF IRISIN D. R. Maddox, A. M. Brown, & D. R. Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. Obesity is an epidemic in today's society with many negative effects, including type II diabetes, heart disease, and stroke. Due to this public health problem, the study of adipose tissue is central to the understanding of metabolic abnormalities associated with the

development of obesity. Irisin is a protein cleaved from the fibronectin type III domain containing 5 (FNDC5) protein in the skeletal muscle. It affects white adipose tissue by acting as a metabolic mediator. This effect is known as the “browning” of white fat where white adipose tissue is physiologically converted into brown adipose tissue. We applied computational techniques to investigate the characteristics of irisin by studying proteins homologous to irisin. Homology modeling with Modeller, Dali Server/LOMETS structure searches, and molecular dynamics simulations performed with GROMACS have been utilized to study the way irisin acts in comparison to the well-known homologous proteins. As a preliminary result, irisin acts much like the neural cell adhesion molecule 2 (NCAM2), which is thought to bind to the alpha-2,8-polysialic acid surface receptor in neural cell membranes. NCAM2 shares secondary and tertiary structure similarity to irisin, which suggests the proteins may share surface receptor types. Exploring the structural characteristics of the NCAM2 receptor may improve understanding of the role of irisin in metabolism.

TOWARDS THE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE HFQ HOMOLOG FROM *AQUIFEX AEOLICUS*. Kimberly Stanek, Jennifer Patterson, & Cameron Mura, Department of Chemistry, University of Virginia, Charlottesville, VA 22904. Bacterial survival requires careful regulation of mRNA turnover. The host factor ‘Hfq’ is a bacterial RNA chaperone that functions in mRNA turnover and related pathways, by modulating mRNA-sRNA interactions. As the bacterial branch of the ‘Sm’ superfamily, Hfq homologs tend to assemble into cyclic oligomers that associate with RNA. An Hfq has been identified in the genome of the deep-branching thermophile *Aquifex aeolicus* (*Aae*), but little is known about its structure or activity. Earlier work has demonstrated that *Aae* Hfq cannot complement Hfq-deletion strains of *Salmonella enterica*, implying that different species have distinct RNA-binding profiles in vivo. To elucidate the structure and function of *Aae* Hfq, the protein has been over-expressed, purified, and crystallized; *Aae* Hfq oligomers have also been studied by cross-linking. In addition, *Aae* Hfq has been shown to co-purify with small endogenous RNAs. The binding of *Aae* Hfq to FAM-U6 and FAM-A18 has been studied via fluorescence polarization assays and binding constants have been determined.

COMPARISON OF ATOMISTIC MOLECULAR MECHANICS FORCE FIELDS FOR SIMULATING THE ALZHEIMER'S AMYLOID  $\beta$ -PEPTIDE. S. R. Gerben, J. A. Lemkul, & D. R. Bevan, Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0308. Macromolecular function arises from structure, and many diseases are associated with misfolding of proteins. Molecular simulation methods can augment experimental techniques to understand misfolding and aggregation pathways with atomistic resolution, but the quality of these predictions is a function of the parameters used for the simulation. There are many biomolecular force fields available, but most are validated using stably folded structures. Here, we present the results of molecular dynamics simulations on the amyloid  $\beta$ -peptide (A $\beta$ ), whose misfolding and aggregation give rise to the symptoms of Alzheimer's disease. Because of the link between secondary structure changes and pathology, being able to accurately model the structure of A $\beta$  would greatly improve our understanding of the disease, an outcome with implications for studying other

protein misfolding disorders. To this end, we compared five popular atomistic force fields (AMBER03, CHARMM27, GROMOS96 53A6, GROMOS96 54A7, and OPLS-AA) to determine which could best model the structure of Ab. By comparing secondary structure and calculated NMR shifts to available experimental data, we conclude that AMBER03 and CHARMM27 over-stabilize helical forms of Ab, whereas OPLS-AA, GROMOS96 53A6, and GROMOS96 54A7 produce very similar results that agree well with experimental observables.

CHARACTERIZATION OF UDP-ARABINOPYRANOSE AS A SUBSTRATE OF EUKARYOTIC UDP-GALACTOPYRANOSE MUTASES. William T. Ryan, Karina Kizjakina & Pablo Sobrado, Department of Biochemistry, Virginia Tech., Blacksburg VA 24061. UDP-Arabinofuranose is a precursor for L-arabinofuranose, an important constituent in plant cell walls. In plants, UDP-arabinopyranose (UDP-Arap) is converted into UDP-arabinofuranose (UDP-Araf) by the enzyme UDP-arabinopyranose mutase (UAM). UDP-galactopyranose mutases (UGMs) interconvert UDP-galactopyranose (UDP-Galp) into UDP-galactofuranose (UDP-Galf), a reaction important for virulence in the human pathogen *Aspergillus fumigatus*. The substrates for both classes of mutases are very close in their chemical composition, yet these enzymes perform their function through seemingly different mechanisms. While UGM family requires a flavin cofactor for catalysis, UAM does not require this cofactor for activity. Herein, we investigate the activity of eukaryotic UGMs with UDP-Arap/f as a substrate. It was determined that *A. fumigatus* UGM was able to utilize UDP-Araf only when the flavin was in the reduced state. A  $k_{cat}$  value of  $0.013 \text{ s}^{-1}$  was determined. This is ~5000-fold lower than with UDP-Galf. The  $K_m$  value for UDP-Araf was  $65 \mu\text{M}$ , which is 1.72-fold lower than for UDP-Galf. Fluorescence polarization binding assay revealed that the  $K_d$  value for UDP-Arap was  $347 \mu\text{M}$  and  $1179 \mu\text{M}$  in the reduced and oxidized state, respectively. Supported by NIH grant R01 GM094469.

SOLUTION CONDITIONS AND OXIDATION STATE OF METHIONINE-35 AFFECT THE AGGREGATION PROPERTIES OF THE AMYLOID  $\beta$ -PEPTIDE (1-40). A. M. Brown, J. A. Lemkul, N. Schaum, & D. R. Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. The amyloid  $\beta$ -peptide ( $\text{A}\beta$ ) is a 40-42 residue peptide that is the principal toxic species in Alzheimer's disease (AD). With AD being the primary causes of senile dementia in the United States, understanding the mechanism by which  $\text{A}\beta$  aggregates is essential in therapeutic design. The conversion of  $\text{A}\beta$  from a helical configuration in the membrane to a  $\beta$ -strand configuration is essential for aggregation. The oxidation of methionine-35 (Met35) to the sulfoxide form (Met35<sup>ox</sup>) has been identified as potential modulator of  $\text{A}\beta$  aggregation. Experimental studies disagree on the role Met35<sup>ox</sup> plays in  $\text{A}\beta$  neurotoxicity, which may be due to inconsistent solution conditions (pH, buffer, temperature). We applied atomistic molecular dynamics (MD) simulations as a means to probe the dynamics of the 40-residue alloform of  $\text{A}\beta$  ( $\text{A}\beta_{40}$ ) containing Met35 or Met35<sup>ox</sup>. We found that Met35 oxidation decreases the  $\beta$ -strand content of the C-terminal hydrophobic region (residues 29-40), with a specific effect on the secondary structure of residues 33-35, thus potentially impeding aggregation. Further, there is an important interplay between oxidation state and solution conditions, with pH and salt concentration augmenting the effects of oxidation. The results presented here serve to



rationalize the conflicting results seen in experimental studies and provide a fundamental biophysical characterization of A $\beta$ <sub>40</sub> dynamics in both reduced and oxidized forms. Ultimately, understanding the characteristics of A $\beta$  that are important for aggregation, such as the oxidation state of Met35, can provide insight into the biochemical mechanism of AD.

CHARACTERIZATION OF AN IMP CYCLOHYDROLASE IN *HALOFERAX VOLCANII*. Michelle L. Pasier & Cathy A. Sarisky, Dept. of Chem., Roanoke College, Salem VA 24153. Purine biosynthesis is a fundamental metabolic pathway present in essentially all life forms. The domain Archaea, unlike bacteria and eukaryotes, does not universally conserve enzymes across this pathway. Archaeal purine biosynthetic enzymes are also not necessarily homologous to non-archaeal enzymes. The final reaction in *de novo* pathway is the conversion of 5-formamidoimidazole-4-carboxamide ribotide (FAICAR) to inosine-5'-monophosphate dehydrogenase (IMP). In *Haloferax volcanii*, a halophilic archaeon, this reaction is catalyzed by an IMP cyclohydrolase. The genome of *H. volcanii* is sequenced, but the characterization of its genes is not yet completed. The gene HVO\_0011 encodes a protein with a high amino acid sequence similarity to TK0430's, an archaeal IMP cyclohydrolase. HVO\_0011 was inserted into *E. coli* cells in the vector pMal-c5e. These cells were induced to express the protein of interest. This protein was extracted from the induced cells by sonication and purification. The protein of interest was then separated from *E. coli* proteins using size exclusion and placed in a buffer simulating *H. volcanii* cellular conditions. An assay of the protein of interest and FAICAR was shown to produce IMP by its presence in the assay solution by HPLC chromatography. This confirms the protein encoded by HVO\_0011 as an IMP cyclohydrolase.

DISABLED-2 (DAB-2) MODULATES PLATELET- CANCER CELL INTERACTIONS THROUGH ITS SULFATIDE BINDING DOMAIN. K. J. Andreano, X. Fu, D. G. S. Capelluto, C. V. Finkielstein. Dept. of Biol. Sci., Virginia Tech, Blacksburg, VA 24060. Platelets' role in cancer progression and metastasis has been attributed to platelet-mediated enhancement of tumor cell survival, extravasation and angiogenesis. Correlations exist between the tumor cells' ability to aggregate platelets in vitro and their metastatic potential in vivo that it is manifested as a hypercoagulable state. We have identified a tumor suppressor (Dab-2) that is released upon platelet activation and that modulates the extent of blood clotting. Our results show Dab-2 is released from platelet alpha-granules to the membrane surface where it binds to the integrin receptor, inhibiting platelet aggregation. Dab-2 binding to integrins is modulated by sulfatides, glycosphingolipids that accumulate in platelet membrane and effectively compete for Dab-2. We found that the N-terminal region of Dab-2 binds to sulfatides through two conserved sulfatide-binding sites. Upon activation, sulfatides protect Dab-2 from thrombin cleavage, facilitate its internalization and modulate the surface expression of P-selectin, a coagulation protein needed for stabilization of platelet aggregates. P-selectin mediates tumor cell adhesion to vascular endothelial cells and the interaction between activated platelets and cancer cells (emboli) during metastasis. Our data show that Dab-2/sulfatide recognition influences the stability of platelet aggregates heterotypically with cancer cells through sulfatide binding and, indirectly, by controlling P-selectin levels. Thus, we hypothesize that

manipulation of Dab-2 function in response of platelet activation will impact emboli formation offering an alternative route for therapeutic strategies aimed to control metastatic processes.

THE ROLE OF ENTROPY IN THE REGULATION OF 3-PHOSPHOGLYCERATE DEHYDROGENASE. C. Meehan, A. Magnuson, C. Roach & E. Bell, Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173. Proteins can take on an array of conformations determined by regional flexibility of the protein. This may play critical roles in catalysis and allosteric regulation. The relationship between structure and function was studied to determine the contributions particular amino acids in 3phospho-glycerate dehydrogenase (3pGDH), may play in flexibility and function of the protein. Based upon available crystal structures a number of amino acids were identified by analysis of the temperature factors that may show altered flexibility in response to serine binding. Additionally, in conjunction with analysis of the temperature factors of bound cofactor (NAD(H)) we observe that two of the 4 central loops in the tetramer structure are more flexible than the other two and that this correlates with increased affinity for the cofactor. This creates an entropy sink that can be used to harness flexibility changes for catalysis and allosteric regulation. Various point mutations of specific amino acid residues involved in this "entropy sink" were created and the expressed proteins were purified using Ni-NTA affinity chromatography. Preliminary results suggest significant effects on activity. Our observations suggest the presence of an entropy sink, which contributes to cofactor binding cooperation and possibly catalysis. This work is supported by NSF Grant MCB 0448905 to EB.

ANS AS A PROBE OF LIGAND INDUCED CHANGES IN OLIGOMERIC DEHYDROGENASES. G. Kingdom<sup>1</sup>, S-H. Kim<sup>2</sup>, C. Guzman<sup>1</sup>, C. Metz<sup>1</sup> and E. Bell<sup>1</sup>, <sup>1</sup>Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173 & <sup>2</sup>Maggie Walker Governor's School, Richmond, VA 23220. 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence in the presence or absence of 3 oligomeric dehydrogenases has been used to assess ligand-induced changes in conformational plasticity. We have explored cofactor and carboxylic acid induced changes in various dimeric malate dehydrogenases including porcine mitochondrial and cytosolic forms and watermelon glyoxysomal forms. With 3-phosphoglycerate dehydrogenase, a tetrameric enzyme found in higher eukaryotes including humans and in prokaryotes such as E. coli, we have compared the E coli and human forms using NSD mutants (containing the cofactor and substrate binding domains and the critical cofactor domain dimerization interface where a conserved tryptophan crosses the interface). Human NSD enhances ANS fluorescence more than E coli NSD suggesting increased conformational plasticity. Alpha-ketoglutarate appears to enhance indicators of conformational flexibility. With hexameric glutamate dehydrogenase, the effects of the substrate Glutamate and a non catalytically active analog glutarate on ANS fluorescence suggest that, while glutarate has little effect on the overall conformational plasticity of the enzyme, reduced cofactors result in a greatly enhanced blue shift in ANS fluorescence, suggesting enhanced binding to newly exposed hydrophobic regions of the protein. Funded in part by NSF Grant MCB 0448905 to EB.

THE ROLE OF CONFORMATIONAL FLEXIBILITY IN NITRIC OXIDE SYNTHASE. Rachel Jones<sup>1</sup>, & Ellis Belf<sup>2</sup>, <sup>1</sup>Clover Hill High School, Midlothian VA, 23112, <sup>2</sup>Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173. Nitric Oxide Synthase (NOS) plays a critical role in a variety of signaling pathways and responses to cellular challenge. Although the enzyme is well studied, the role of conformational flexibility in Nitric Oxide Synthase (NOS) activity has received little attention. NOS exists in three isoforms, inducible Nitric Oxide Synthase (iNOS), associated with malignant disease; neuronal (nNOS), and endothelial (eNOS). Each consists of the N-terminal oxygenase domain which binds Arginine and NADPH and the C-terminal reductase domain that binds flavin adenine dinucleotide, and calmodulin. To understand the role of conformational flexibility, experiments using 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence, and multi-wavelength collisional quenching with differently charged quenchers were conducted in the presence/absence of Arginine to determine how exposed hydrophobic regions of the protein were changed in iNOS. The data suggests that Arginine increases the number of ANS binding sites per protein molecule; implying an increase in iNOS non-polar regions. The collisional quenching data suggests that Arginine increases the exposure of non-polar regions, suggesting that Arginine increases the flexibility of the protein. Analysis of the amino acid sequence of iNOS suggests that these changes in flexibility could be related to the regulation of disordered regions of the protein. A comparison of the effects of a number of inhibitors of iNOS on both ANS fluorescence or the multi-wavelength collisional quenching suggest different structural basis for their inhibitory effects.

EXPLORING THE STRUCTURAL BASIS FOR PIP<sub>2</sub> SENSITIVITY OF INWARDLY RECTIFYING POTASSIUM CHANNELS. Trevor Larry, Kalen Hendra, Arco Paul & Linda M. Bolland, University of Richmond, Department of Biology, Richmond, VA 23114. All vertebrate inwardly rectifying potassium (Kir) channels are activated by phosphatidyl-inositol 4,5-bisphosphate (PIP<sub>2</sub>). Dynamic changes in PIP<sub>2</sub> concentrations impact how Kir channels control the cellular resting membrane potential. This project investigates a novel Kir channel (AqKir) that was cloned from the marine sponge *Amphimedon queenslandica*. Study of the sponge channels may provide insight into key changes in structure and function that occurred during animal evolution. To probe the requirement for PIP<sub>2</sub> in the maintenance of AqKir, we reduced PIP<sub>2</sub> concentrations in three ways: (1) application of wortmannin (at mM concentrations) to inhibit the kinase that phosphorylates PI to PIP; (2) activation of a co-expressed muscarinic acetylcholine receptor to activate a G protein-coupled phospholipase C; (3) activation of a co-expressed voltage-sensing phosphatase (CiVSP). The results confirm that PIP<sub>2</sub> regulates AqKir but the sponge channel requires much less PIP<sub>2</sub> to remain active when compared to vertebrate homologs. We used mutagenesis to identify two key residues that are important for higher sensitivity to PIP<sub>2</sub> of the vertebrate Kir channels.

TO TRANSLATE OR NOT TO TRANSLATE: HOW DED1, A DEAD-BOX ATPASE, REGULATES MRNA TRANSLATION. Angie Hilliker, Dept. of Biology, University of Richmond, Richmond, VA 23173. Translation regulation is important, especially during stress, in neurons, and in early development, where many mRNAs

are stored in translationally repressed states. These repressed mRNAs can be returned to translation, but this process is poorly understood. We have previously shown evidence that the RNA helicase, Ded1, performs two sequential functions to return a repressed mRNA back to translation in an ATP-dependent manner. First, Ded1 promotes the formation of a “pre-48S mRNP” that includes the initiation factors eIF4F and Pab1. Then, Ded1 hydrolyzes ATP, allowing the mRNA to become translated. We hypothesize that Ded1 acts as an ATP-dependent switch to return mRNAs back to the translating pool. We show that Ded1 interacts directly with eIF4G, while others have shown that the mammalian ortholog of Ded1, DDX3, interacts with both eIF4E and Pab1. We find that Ded1 helps recruit eIF4E and Pab1 to the m7G cap *in vitro*. We are currently testing the roles of polyA binding protein and the polyA tail in helping Ded1’s first role in forming the pre-48S intermediate. Additionally, genetic screens have revealed factors that may modulate Ded1’s first or second function via post-translational modifications. These studies will help us understand how Ded1 is regulated, which will illuminate how mRNAs return to from a repressed state back into translation.

**INDUCTION OF APOPTOSIS BY NONSTRUCTURAL PROTEINS OF THE SINDBIS VIRUS IN XENOPUS LAEVIS EMBRYOS.** Kaitlyn Childs, Jacob Graham, Kevin M. Myles, & Carla V. Finkielstein, Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Viral infections are of interest as is related to virotherapy and host-virus interaction studies for mechanism construction that may result in knockout therapies to reduce or diminish virulence. *Sindbis* virus, an Alphavirus of the Togaviridae family, is transmitted by mosquitos; the endogenous strain is opportunistic, infecting immunocompromised individuals, elders and young children. The viral genome is divided into nonstructural (ns) and structural open reading frames. The nonstructural region encodes a polyprotein consisting of nsP1, nsP2, nsP3, and nsP4. In this study, we have found that the nonstructural protein nsP3 has the ability to induce apoptosis in an heterologous system and that this property is restricted to its N-terminus domain. Interestingly, whereas the nonstructural polyprotein is able to trigger apoptosis in *Xenopus* embryos when a polycistronic mRNA is injected in one-cell stage, neither nsP1, 2 nor 4 was able to accomplish this form of cell death on their own. Apoptosis was confirmed by measuring caspase-3 activity, visualization of membrane blebbing, and cleavage of cyclin A2, a known caspase substrate in *Xenopus* early embryogenesis. Next, we investigated whether nsP3 pro-apoptotic activity was inhibited by interacting with anti-apoptotic members of the Bcl-2 family. Thus, *Xenopus laevis* embryos were injected with various nsP3 constructs in one-cell, collected at different times before MBT and will be analyzed for binding by immunoprecipitation. We hope to elucidate the mechanism behind apoptosis induction of the *Sindbis* virus in *Xenopus laevis* embryos in this way.

**DOWN-REGULATION OF THE CIRCADIAN FACTOR PERIOD 2 BY THE ONCOGENIC E3 LIGASE Mdm2: EVIDENCE OF THE PRESENCE OF CIRCADIAN AND CELL CYCLE CROSSTALK MECHANISMS.** Jing-Jing Liu, Tetsuya Gotoh, Marian Vila-Caballer, Carlo S. Santos, Jianhua Yang & Carla V. Finkielstein, Integrated Cellular Responses Laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg, VA. The circadian rhythm and cell cycle are the

two main oscillatory systems in cells. How cells sense time and decide what is the best time for growing, proliferating or apoptosis? One possibility is that there are crosstalks between these two systems. Based on the fact that Period 2 (Per2) also plays essential role in DNA damage response, Per2 is supposed to connect circadian rhythm and cell cycle, which makes Per2 work as a tumor suppressor. We found Per2 regulating p53 pathway but little is known about how Per2 itself is regulated. One interesting finding is that independent of transcriptional regulation, overexpressed Per2 protein also oscillates; this implies posttranslational modifications are essential for sustaining Per2 protein oscillation. Per2 binds to Mdm2, a well-known E3 ubiquitin ligase, both *in vitro* and *in vivo*. Mdm2 induces Per2 ubiquitination *in vitro*, but further experiments are needed to verify Mdm2 is an E3 ligase for Per2 *in vivo*.

USE OF ELECTROSTATIC POTENTIALS TO EVALUATE PPAR $\alpha$  FOR DRUG DISCOVERY. F. Buzzalino, N. Lewis & D. Bevan, Dept. of Biochemistry, Virginia Tech 24060. It is estimated that in the United States, approximately 600,000 deaths per year are attributed to cardiovascular disease. Peroxisome proliferator activating receptors (PPARs), transcription factors that pertain to the nuclear receptor family, play an important role in maintaining metabolism. PPAR $\alpha$ , particularly responsible for the catabolism of fatty acids, resides in the liver, heart, kidneys, and skeletal muscle. In recent years Fibrates, synthetic ligands used to treat hyperlipidemia via specific activation of PPAR $\alpha$ , have demonstrated effectiveness in reducing cardiovascular disease. Our goal is to identify unknown ligands that interact with PPAR $\alpha$  in a similar manner as fibrates. Prior to performing studies to identify unknown ligands, an appropriate PPAR $\alpha$  structure must be chosen. In our studies we have analyzed the binding of 11 PPAR $\alpha$  crystal structures with the 11 crystalized agonists via docking experiments (re-docking, and cross-docking). Our analysis of the residue interactions between the 11 PPAR $\alpha$  structures and the agonists reveal that four residues – His440, Tyr314, Tyr464, Ser280 – are associated with agonist binding. Docking of 544 compounds with three PPAR $\alpha$  for the presence of the four residues was performed. The PPAR $\alpha$  structure 2P54 demonstrated to be representative of a structure that can bind varying compounds. Our results lead us to believe that the most appropriate PPAR $\alpha$  structure for docking of unknown ligands is 2P54. Identification of PPAR $\alpha$  selective ligands may help produce fibrates in hopes of reducing the number of casualties associated with cardiovascular disease.

### 2013 Ivey F. Lewis Distinguished Service Award

The Ivey F. Lewis Service Award is the Academy's highest form of recognition of service. First presented in 1956 as the Meritorious Service Award it was renamed in 1966 to honor the first president and "founder father" of the Academy.



**James Patrick O'Brien, Ph.D.**

A Sustaining Member of the Academy, James P. O'Brien, Professor of Psychology at Tidewater Community College, joined VAS in 1973, was the Academy's 71<sup>st</sup> President (1993-94) and was named a Fellow in 1997. Currently, he is the Academy's Delegate to the American Association for the Advancement of Science and the National Association of Academies of Science, Chair of the Fellows Fund Committee, Co-Chair of the Fund Raising Committee, Chair of the Awards Committee, Associate Editor of *Virginia Scientists*, and a member of the Publications Committee. He has served on Council as the Psychology Section Representative since 1977, beginning as the alternate Councilor for three years; in the Psychology Section he held several terms as Vice Chair, Secretary, Editor, and Chair, Director of the Section's Invited Lecturer Series, Program Chair, and Session Chair. Dr. O'Brien was appointed to the Academy's statutory Seat 14 on the Board of Trustees of the Science Museum of Virginia by Governor Timothy Kaine and, for a second term, by Governor Robert McDonnell; he is Chair of SMV's Science Education Committee, a member of the Science Advisory Committee and Executive Committee, and has served on the Strategic Planning Committee, Nominations Committee, and Broad Street Campus

Committee. He is the Board of Trustees' Auditor on the Virginia Outstanding Scientist Selection Committee.

In the 1970s-80s, he sent Calls-for-Papers and invitations to non-members within a 50 mile radius of the annual meeting as Chair of the Membership Committee and prepared media packages for annual meetings as Chair of the Publicity/Public Affairs Committee (ad hoc). Jim has also been a member of the Long Range Planning Committee, the Committee on the Future of the Academy (ad hoc), the 75<sup>th</sup> Anniversary Committee (ad hoc), the Junior Academy of Science Committee, Nominations and Elections Committee, and was Director of the Academy's Biennial Leadership Retreats. As President-Elect, he was Program Chair of the 1993 Annual Meeting at Old Dominion University and also solicited exhibitors for several annual meetings. Council named him Founding Editor of *Virginia Scientists* in 1991, which he has variously served as Editor, Associate Editor, and Assistant Editor.

Over the past 40 years, he has co-authored scores of VAS research papers with approximately 80 of his honors students, colleagues, and students, presenting in the Sections on Psychology, Medical Sciences, Engineering, and Education. Jim was Director of the 75<sup>th</sup> Anniversary Campaign in which donors generously contributed \$13,000 to the Academy. With the assistance of Jim and Lisa Martin and then-SMV Director Walter R. T. Witschey, he negotiated and directed the move of the Academy's Central Office from the University of Richmond to the Science Museum of Virginia. He established or re-established the following Academy Sections: Computer Science (with Gregory C. Cook of Tidewater Community College), Archaeology (with J. Mark Wittkofski of the Council of Virginia Archaeologists), Natural History and Biodiversity (with the Virginia Natural History Society and the assistance of Elsa Falls and Carolyn Conway), and Geography (with Stephen E. Wright of James Madison University).

Trained in engineering and environmental psychology, his current research in social psychology focuses on independence and conformity. In the Human Factors and Ergonomics Society, he was Co-Chair of the Public Affairs Committee, South Regional Editor, a member of the Education Committee and Council of Chapter Presidents; he was President of the Tidewater HF&ES Chapter, 1983-84. He joined the American Association for the Advancement of Science in 1970, was nominated Chair of Section Y in 2012, and was named a AAAS Fellow in 2011. Currently, Jim is also a member of the Association for Psychological Science, Virginia Psychological Association, Virginia Academy of Academic and Applied Psychology, and Psychology Teachers at Community Colleges (American Psychological Association affiliate), and Virginia Community College Association. He is a member of Old Dominion University's Darden College of Education Advisory Board, Dominion Energy Options Advisory Board, and The Talbot Hall Foundation Board of Directors.

Dr. O'Brien was named "Virginia Professor of the Year" by the Carnegie Foundation for the Advancement of Teaching and the Council for the Advancement and Support of Education (2010) and received the Outstanding Faculty Award from the State Council of Higher Education for Virginia (2011). The Virginia Community College System twice awarded Jim Chancellor's Commonwealth Professorships (2003-05, 2007-09); in 2007, VCCS named him the inaugural recipient of the Chancellor's Award for Teaching Excellence. He joined the faculty at Tidewater Community College in 1972; TCC has recognized him as Professor of the Year (2006-07), TCC-Charles University (Prague) Faculty Exchange Scholar (1999), and as recipient of the

International Education Award (2000), Outstanding Faculty Advisor Award (1999-2000), and the George B. Pass Memorial Distinguished Service Award (1994). He has also been an adjunct faculty member at Virginia Commonwealth University and the University of Virginia. Dr. O'Brien is listed in Marquis' *Who's Who in America* (2011-present), *Who's Who Among America's Teachers* (1994-97), and *Who's Who Among College and University Students* (1966, 1971, 1972).

He received his B.A. in Psychology from the University of Richmond (1966, Scabbard and Blade, Phi Delta Theta), and the M.S. in General-Applied Psychology from Virginia Commonwealth University (1969, Psi Chi). He earned his doctorate in Human Factors Psychology at The Catholic University of America (1977, President's Scholar). Other post-baccalaureate studies include T. C. Williams School of Law (University of Richmond, Phi Delta Phi), University of Richmond Graduate Arts & Sciences, University of Michigan Human Factors Engineering Summer Conferences, George Washington University School of Business (Delta Nu Alpha), George Washington University School of Medicine, American University Schools of Public Policy and Education, University of Virginia Tidewater Extension, and Virginia Tech Hampton Roads Extension.

Jim and Mary Louise are members of the Episcopal Church of the Ascension, Norfolk and are the proud parents of Christopher O'Brien of Norfolk, Andrew and wife Alicia O'Brien of Annapolis, and Marquis Evans of Randolph-Macon College.



**2013 FELLOWS OF THE ACADEMY**

From active membership, there shall be a body of scholars known as "Fellows of the Virginia Academy of Science." Fellows are selected because of their contribution to science in one or more of the following ways: (a) outstanding scientific research, (b) inspired teaching of science, or (c) significant leadership in the Academy. A Fellow must be nominated by at least three Members of the Academy. Nominations should be sent to:

Executive Officer  
Virginia Academy of Science  
Science Museum of Virginia  
2500 W. Broad Street  
Richmond, Virginia 23220.

The Executive Officer will verify receipt of nomination and membership status and forward the nominations to the Chair of the Awards Committee. Final selection is by majority vote of the Academy Council. The following members were elected as Fellows of the Virginia Academy of Science for 2013.



**Thomas W. Haas, Ph.D.**

Dr. Thomas W. Haas received a B.S. degree in mechanical engineering from SUNY Buffalo, a M.S. degree in engineering mechanics from Pennsylvania State University, and M.A. and PH.D. degrees in Aerospace and Mechanical Science from Princeton University in the Polymer Materials Program.

A member of the Academy since 1985, Dr. Haas was elected President Elect of the Academy and became the 76th President at the 1998 Annual Meeting, having served as both Secretary and Chair of the Engineering Section. Tom chaired the Local

Arrangements Committee for both the 1989 and 1996 Annual Meetings held at Virginia Commonwealth University. He currently serves as Councilor for the Biomedical and General Engineering Section.

Dr. Haas joined Virginia Commonwealth University in 1983 as Professor and Director of the Commonwealth Graduate Engineering Program, a consortium of Virginia engineering schools offering graduate programs leading to a master's degree in engineering delivered by interactive television from the University of Virginia, Virginia Tech, George Mason University, and Old Dominion University. He also served as Associate Dean for Academic and Faculty Affairs of the new VCU School of Engineering from 1995 to 1999.

In addition to having teaching experience at the University of Illinois, Stevens Institute of Technology, and the New Jersey Institute of Technology, he has held research, development, and engineering positions at Philip Morris USA, ICI Americas, American Standard, Inc, and AT&T Bell Laboratories.

Tom is also a Past President of the Society of Plastic Engineers, an international society of 30,000 members. Prior to assuming the post of Society President in 1982, he was a member of the SPE Executive Committee and was Secretary, Second Vice President, and President-Elect. He also served for ten years as a member of the Board of Directors of the Accreditation Board for Engineering and Technology (ABET) representing the Society of Plastic Engineers. A Distinguished Member of SPE, he is also a Fellow of the Plastics and Rubber Institute (now the Institute of Materials, Minerals, and Mining) in the United Kingdom and a member of the American Chemical Society (ACS), the Society of Rheology (SOR), and the American Society of Mechanical Engineers (ASME), having served as the 2000-2001 Chair of the Central Virginia ASME Section. Tom is currently a past chair and Executive Director of the Richmond Joint Engineers' Council (RJEC).

Tom's research expertise is in plastics and biopolymers. His research interests include crystallization and morphology, mechanical and physical properties, plastics in medical devices, electro-spinning of biopolymers and polymer processing. He has a number of papers, patents, and publications pertaining to plastics and polymers.



**Michael H. Renfroe, Ph.D.**

Dr. Michael H. Renfroe, an active member of the Academy since 1986, served as Treasurer, Secretary, Vice-President, and President-Elect the Academy before being elected the 89<sup>th</sup> President at the 2011 Annual Meeting. He has also served as Vice-Chair, Secretary and Chair of the Botany Section. He currently serves the Virginia Flora Committee and the Long-Range Planning Committee as well as the VAS Visiting Scientists Program Speakers Bureau.

Dr. Renfroe joined the Biology Department at James Madison University (JMU) in 1986 as an assistant Professor and became full Professor in 2003. Before joining the faculty at James Madison University, he served a Teaching Associate at both North Carolina State and Ohio State Universities. In addition, he also held research positions at the associate, fellow, and post-doctoral levels at Ohio State University, the University of Georgia, Yale University, and Texas A&M University. His education degrees include a B.S. from North Carolina State University, a M.S. from Ohio State University, and both a Masters in Philosophy and a Ph.D. from Yale University.

Dr. Renfroe is both a master educator as well as a dedicated researcher. During his career at JMU, he has taught over 25 different courses while continuing his research with his students. In addition, he has authored or co-authored over 60 publications, proceedings, and abstracts. He and/or his students have presented over 60 papers or posters including 29 at the Botany Section of VAS. Since becoming active with the Academy in 1986, he and/or his students have presented papers in the Botany Section at every VAS Annual Meeting. His profession service is diverse ranging from serving as a member of the Flora of Virginia Project Advisory Board and other botanically research-oriented organizations to organizations involved with science education at childhood to high school to college levels. He is also active as a reviewer for manuscripts for scientific journals and science textbooks.

Here is how Dr. Renfroe describes his dedication to education, research, and the role VAS plays in his professional life: "I started work at JMU in 1986. In addition to teaching biology to majors, I have been involved in teaching non-majors in the general education program. I also have been involved in teaching in-service and pre-service science teachers. For a number of years I was a teacher in a regional Governor's School program during summers working with high school students whose studies focused on environmental themes. Some of my courses for majors include Plant Cell and Tissue Culture and an economic botany course. My research has focused on plant tissue culture and antioxidants in plant-based foods and beverages. My students and I have conducted research on propagation of Virginia round-leaf birch through bud and meristem culture. We also have conducted research on *Saintpaulia rupicola*, a critically-endangered species of African violet. We have developed methods of rapid propagation from leaf explants and have developed methods for production and short-term storage of synthetic seeds for African violets. We have examined the antioxidant content of fresh and processed forms of fruits such as blueberries and strawberries. We have also extensively studies tomatoes and tomato products, teas, spices, and wines. VAS helped launch my research career at JMU by providing grants that supported my research. VAS is a great training ground for undergraduate and graduate students learning to make professional presentations [and] provides an incredible opportunity to network and get to know fellow scientists from across the state. VAS has been a tremendous supporter of scientific research in Virginia and has provided research funds for an incredible number of scientists over the years. Personally, I think that every scientist in the Commonwealth should want to be a member of VAS so that we can demonstrate the power of science to improve our lives and to disseminate the newest information to make a more informed citizenry."



**Werner Wieland, Ph.D.**

Werner Wieland, Professor of Biological Sciences at University of Mary Washington, has been elected a Fellow of Virginia Academy of Science by VAS Council at its March 2013 meeting.

A 1973 graduate of Virginia Commonwealth University, Wieland received the Master of Science degree in biology from VCU in 1976, where he was a faculty research assistant and adjunct faculty member. He worked as a Biological Technician, USFWS, Alabama Fisheries Research Unit, in Auburn, Alabama, in 1977-1978, and earned his doctorate in zoology from Auburn University in 1983, where he was President of Auburn University Student Chapter, American Fisheries Society.

The focus of Dr. Wieland's research is fish stocks and their ecosystems in Virginia tributaries, the Tennessee River, and the Gulf of Mexico. The American Fisheries Society designated him a Certified Fisheries Scientist in 1985 and a Certified Fisheries Professional in 2001 and 2007. He has worked as a Technical Consultant for a variety of clients, including the Chesapeake Bay Foundation.

He is a member of Sigma Xi, the American Fisheries Society, the Southeastern Fishes Council, the Association of Southeastern Biologists, and the American Society of Ichthyologists and Herpetologists. He has served on various committees in these organizations including a term as President of the Virginia Chapter of the American Fisheries Society in 1996. He has also served as a panelist for the National Science Foundation Graduate Research Fellowship Program.

As Professor of Biological Sciences at Mary Washington, Dr. Wieland has mentored numerous students engaged in undergraduate research, and some of his students have presented at the Annual Meeting of VAS. He was Chair of his department from 2002-2011 and honored with a Distinguished Faculty Achievement Award by the Commonwealth of Virginia in 2001 and Mary Washington's Mary Pinschmidt Award in 2000.

He has been an active member of VAS since 1984 and has held a variety of leadership positions in the Academy. Initially, Werner was a member of the Biology Section and held every office within the section. He was an advocate for the formation of the Natural History and Biodiversity Section, started in 1994; he has held every office in this section and, on a number of occasions, held two positions simultaneously.

In May 2005, Werner was elected VAS Vice-President and served as Program Chair for the 84<sup>th</sup> Annual Meeting in 2006. He was President of the Academy in 2007-2008. He has served as Editor of the *Virginia Journal of Science* since 2002 and currently is a member of the Long Range Planning Committee and the Publications Committee.

Dr. Wieland explains, "My first experience giving a presentation at a professional meeting was at the VAS Annual Meeting in 1972, held at Lexington (VMI). Upon returning to Virginia I renewed my membership in VAS and have since encouraged all my students to present their research at the annual meeting as a first step toward pursuing a career in the biological sciences. The annual meeting provided an excellent first experience for me. The Academy brings together colleagues from throughout the Commonwealth and through the *Virginia Journal of Science* provides an excellent periodical for the dissemination of their scholarly endeavors."

In recognition to his many contributions within biology, his mentoring of countless students, and his long-time service to VAS, the Academy is pleased to name him a Fellow.

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